STATISTICS OF NEAREST-NEIGHBOUR DIPOLE AND EXCHANGE INTERACTIONS

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Summary

High-temperature partition functions for nearest-neighbour dipole and exchange interactions on the simple cubic lattice are calculated as far as the terms in $(1/kT)^8$. The results for the dipole case are used in a qualitative discussion of some aspects of the theory of polar liquids and solutions. The principal conclusions are that slow convergence probably makes the power series approach unsatisfactory in the theory of highly polar liquids, and even more unsatisfactory in the theory of solutions containing highly polar components. A simple evaluation of the averages over orientations of dipoles required in this and similar work is given in Appendix I.

I. Introduction

In recent theoretical studies of polar liquids and solutions (Pople 1952, 1953; Barker 1953) the partition function for a lattice array of interacting dipoles has been evaluated in a power series in (μ^2/kTV), where μ is the dipole moment and V the volume per dipole. Because of the long range of the dipole interactions it is exceedingly laborious to calculate more than the first few terms of the series, with the result that the conditions under which the series is rapidly convergent or even convergent are not known. Since these questions are obviously of fundamental importance it appeared worth while to attempt to study them in connection with a simplified model, namely, that in which the interactions are restricted to nearest neighbours. While this simplification must change the quantitative features, the qualitative aspects should not be greatly affected. Furthermore the simplified model is of considerable interest in its own right, and some of the mathematical techniques developed are of general application in this kind of problem.

The partition function for a simple cubic array of dipoles, with interaction between nearest neighbours only, has therefore been evaluated as far as the term in $(1/kT)^8$. The similar problem in which the interactions are of exchange type has been treated to the same approximation, and as a by-product of the calculation the results of Ter Haar (1952) on the Ising model are rederived. The dipole interaction calculation has also been carried through for the case in which there are two kinds of dipole, with different dipole moments; these results are used to discuss some aspects of the theory of solutions.

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II. The Model and the Method of Calculation

Consider a simple cubic array of dipoles (this convenient term will be used even when exchange or Ising interactions are under consideration). The configuration of a single dipole is expressed in the case of dipole and exchange interactions by a unit vector **E** which takes all orientations with equal *a priori* probability, and in the Ising case by a quantity ε which takes the values $+1$ and -1 with equal *a priori* probability. The expression for the energy of the array in a given configuration has the following forms:

(i) *Dipole Interaction*

$$U=\frac{\mu^2}{V}\Sigma\mathbf{E}_i \,.\, \mathbf{T}_{ij} \,.\, \mathbf{E}_j, \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where the dyadic $\mathbf{T}_{ij}$ is given in terms of the unit vector **a** in the direction joining the lattice sites i and j by

$$\mathbf{T}_{ij}=(1-3\mathbf{aa}), \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

and Σ means sum over all nearest-neighbour pairs, μ is the dipole moment, and V the volume per dipole.

(ii) *Exchange Interaction*

$$U=2J\Sigma\mathbf{E}_i \,.\, \mathbf{E}_j, \quad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

where J is a constant.

(iii) *Ising Model*

$$U=L\Sigma\varepsilon_i\varepsilon_j, \quad \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

where L is a constant.

The partition function is given in each case by

$$Q=\langle \mathrm{e}^{-U/kT}\rangle, \quad \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

where the angular brackets mean " average over all orientations of all the vectors $\mathbf{E}_i$ " in the dipole and exchange cases, and " average over the values ± 1 of all the quantities ε_i " in the Ising case. The general method is to expand the exponential and evaluate the *a priori* averages of various powers of the energy

$$Q=1-\frac{\langle U\rangle}{kT}+\frac{\langle U^2\rangle}{(kT)^2 2!}-\ldots., \quad \ldots\ldots\ldots (6)$$

This procedure leads to power series in the quantities

$$\alpha=\frac{\mu^2}{kTV},$$

$$\beta=\frac{2J}{kT},$$

$$\gamma=\frac{L}{kT},$$

respectively.

The average of the nth power of the energy U can be expressed as a sum of contributions from simple geometric figures. Thus in the expansion of $\langle U^4 \rangle$ for dipole interactions, one finds terms of the kind

$$\left(\frac{\mu^2}{V}\right)^4 \cdot \frac{4!}{1!1!1!1!} \cdot \langle (\mathbf{E}_1 \cdot \mathbf{T}_{12} \cdot \mathbf{E}_2)(\mathbf{E}_2 \cdot \mathbf{T}_{23} \cdot \mathbf{E}_3)(\mathbf{E}_3 \cdot \mathbf{T}_{34} \cdot \mathbf{E}_4)(\mathbf{E}_4 \cdot \mathbf{T}_{41} \cdot \mathbf{E}_1) \rangle,$$

corresponding to the first geometric figure shown in Table 1, and of the kind

$$\left(\frac{\mu^2}{V}\right)^4 \cdot \frac{4!}{2!2!} \cdot \langle (\mathbf{E}_1 \cdot \mathbf{T}_{12} \cdot \mathbf{E}_2)^2 (\mathbf{E}_2 \cdot \mathbf{T}_{23} \cdot \mathbf{E}_3)^2 \rangle,$$

corresponding to the second and third geometric figures shown in Table 1. Any term in which one of the $\mathbf{E}_i$ appeared raised to an odd power would give zero on averaging ; thus one needs to consider only *closed* figures like those in Table 1.

TABLE 1

CONTRIBUTIONS TO $\langle U^4 \rangle$

Figure	Number of Figures	Multinomial Factor	Dipole Average	Exchange Average	Mixture Factor
	$3N$	4!	1/9	1/27	$(1)^4$
	$3N$	4!/2!2!	8/15	1/9	$(1)^2(2)$
	$12N$	4!/2!2!	2/5	1/9	$(1)^2(2)$
	$3N$	4!/4!	24/25	1/5	$(2)^2$
	$9N^2/2-33N/2$	4!/2!2!	4/9	1/9	$(1)^4$

The *number* of geometric figures of a given type in the whole array can be determined in terms of the total number N of dipoles by a simple counting operation : for the figure K this will be denoted by N_K. Then $\langle U^n \rangle$ can be expressed in the form

$$\frac{\langle U^n \rangle}{(kT)^n} = \sum_K \alpha^n N_K M_K S_D^K \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (7)$$

Here S_D^K, which will be called the " dipole average for the figure K ", is given by

$$S_D^K = \langle \prod_{ij} (\mathbf{E}_i \cdot \mathbf{T}_{ij} \cdot \mathbf{E}_j)^{n_{ij}} \rangle, \quad \ldots\ldots\ldots\ldots\ldots (8)$$

n_{ij} being the number of times that the link i-j appears in the figure K. Also, M_K, which will be called the " multinomial factor ", is given by

$$M_K = \frac{n!}{\prod_{ij} n_{ij}!}. \quad \ldots\ldots\ldots\ldots\ldots (9)$$

The summation $\sum_K$ means " sum for all kinds of closed, not necessarily connected, figures containing n links ".

If there are several kinds of dipoles of moments μ_A, μ_B, . . ., present in fractions x_A, x_B, . . ., and distributed randomly (except for clustering due to the interactions) on the lattice, then instead of the simple factor α^4 one will have $(\sum_A x_A \alpha_A)^4$ for the first figure in Table 1, $(\sum_A x_A \alpha_A)^2 (\sum_A x_A \alpha_A^2)$ for the second and third figures, etc. These factors (" mixture factors ") are shown schematically in Table 1. [$(1)^4$ means $(\sum_A x_A \alpha_A)^4$, $(1)^2(2)$ means $(\sum_A x_A \alpha_A)^2 (\sum_A x_A \alpha_A^2)$, etc.]

Thus to evaluate $\langle U^n \rangle$ one prepares a table like Table 1, multiplies the various factors, and sums. In the table for $\langle U^6 \rangle$ there are 21 entries, in that for $\langle U^8 \rangle$ there are 145 entries. For exchange interactions the dipole averages S_D^K must be replaced by exchange averages S_E^K; a simple procedure for evaluating these averages is given in Appendix I. The corresponding quantities for the Ising model are all equal to unity.

III. Results for Dipole, Exchange, and Ising Interactions

The results will be given in terms of the free energy, which is related to the partition function by the equation

$$F = -kT \ln Q.$$

For the three cases one finds:

(i) *Dipole Interactions*

$$-\frac{F}{NkT} = \alpha^2 + \frac{11}{50}\alpha^4 + \frac{75,436}{297,675}\alpha^6 + \frac{10,052,401}{29,767,500}\alpha^8 + 0(\alpha^{10}), \quad .. \quad (10)$$

$$\alpha = \frac{\mu^2}{kT\overline{V}}.$$

(ii) *Exchange Interactions*

$$-\frac{F}{NkT} = \tfrac{1}{2}\beta^2 + \frac{17}{180}\beta^4 + \frac{527}{8505}\beta^6 + \frac{80,909}{1,701,000}\beta^8 + 0(\beta^{10}), \quad .... \quad (11)$$

$$\beta = \frac{2J}{kT}.$$

(iii) *Ising Model*

$$-\frac{F}{NkT}=\frac{3}{2}\gamma^2+\frac{11}{4}\gamma^4+\frac{271}{15}\gamma^6+\frac{123{,}547}{840}\gamma^8+0(\gamma^{10}), \quad \ldots\ldots \quad (12)$$

$$\gamma=\frac{L}{kT}.$$

The results for the Ising model are in agreement with those of Ter Haar (1952), which provides a check on the identification and counting of the geometric figures.

Using results given by Pople (1952) a comparison can be made between the results for the dipole model with only nearest-neighbour interactions and the model with all-neighbour interactions:

Nearest-Neighbour Interactions:

$$-\frac{F}{NkT}=\alpha^2+0\cdot2200\alpha^4+0\cdot2534\alpha^6+0\cdot3337\alpha^8+0(\alpha^{10}). \quad .. \quad (13)$$

All-Neighbour Interactions:

$$-\frac{F}{NkT}=1\cdot4003\alpha^2-0\cdot551\alpha^3+0\cdot216\alpha^4+0(\alpha^5). \quad \ldots\ldots\ldots \quad (14)$$

TABLE 2

VALUES OF $\alpha=\mu^2/kTV$ AT 20 °C FOR SOME POLAR LIQUIDS

Liquid	α
Chloroform	0·2
Ethyl ether	0·2
Methyl chloride	0·8
Acetone	1·7
Nitromethane	2·5
Nitrobenzene	2·6

Any conclusions that one may draw from the few available terms of these series must be speculative. However, in both cases it seems improbable that the usefulness of the approximation given by the first few terms can extend to values of α greater than unity. If one wished to use the derived series to calculate the entropy this would be even more improbable. In view of the values of α shown in Table 2, it appears that the usefulness of this power series approach in the theory of highly polar liquids has very definite limits. There is a definite need for a method of approximating to the partition function for interacting dipoles which will be valid when the interactions are relatively strong.

IV. Results for Dipole "Mixture"

The general results when there are several kinds of dipole are complicated and not very illuminating in form. For a binary mixture in which one component (B) has dipole moment zero, the free energy is given by

$$-\frac{F}{NkT}=\alpha_A^2x_A^2+\alpha_A^4(-1\cdot5000x_A^4+1\cdot600x_A^3+0\cdot1200x_A^2)$$
$$+\alpha_A^6(3\cdot6214x_A^6-6\cdot8148x_A^5+3\cdot0694x_A^4+0\cdot3695x_A^3+0\cdot0079x_A^2)$$
$$+\alpha_A^8(-11\cdot059x_A^8+28\cdot975x_A^7-24\cdot786x_A^6+5\cdot977x_A^5$$
$$+1\cdot187x_A^4+0\cdot044x_A^3+0\cdot0003x_A^2). \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (15)$$

Using this expression one can calculate the free-energy change on mixing (since the factor $N!/N_A!N_B!$ has not been included in the partition function this will actually be the *excess* free energy of mixing). By differentiating with respect to temperature the excess entropy of mixing can also be calculated.

Table 3

Excess free energy and entropy of mixing for dipole mixture, $\alpha_A^2=0\cdot75$

x_A	F_x^E/RT	Fractional Contribution of α_A^8 Term to F_x^E/RT	S_x^E/R	Fractional Contribution of α_A^8 Term to S_x^E/R
0·1	0·099	0·11	0·227	0·33
0·2	0·175	0·12	0·405	0·35
0·3	0·222	0·13	0·509	0·39
0·4	0·237	0·14	0·525	0·43
0·5	0·225	0·15	0·470	0·50
0·6	0·192	0·17	0·377	0·62
0·7	0·149	0·21	0·281	0·78
0·8	0·100	0·25	0·191	0·92
0·9	0·050	0·24	0·094	0·89

Values of the excess free energy of mixing F_x^E and the excess entropy of mixing S_x^E have been calculated for the value of α_A determined by $\alpha_A^2=0\cdot75$, and are shown in Table 3. This value of α_A was chosen to make the contribution of the term in α_A^8 to the free energy of the pure dipole lattice 10 per cent. of the whole free energy (as calculated using terms up to α_A^8). The additional columns in Table 3 show that if the term in α_A^8 contributes 10 per cent. of the free energy of the pure dipole lattice, it contributes up to 25 per cent. of the excess free energy of mixing and up to 90 per cent. of the excess entropy of mixing. Thus if the use of the first few terms of the series gives a poor approximation to the free energy of the pure dipole lattice, it gives a worse approximation to the excess free energy of mixing and a much worse approximation to the excess entropy of mixing.

One interesting qualitative feature of the results in Table 3 is that the excess entropy of mixing is always positive, which means that the gain of entropy due to the increased freedom of orientation of the dipoles always outweighs the loss of entropy due to " clustering " of the dipoles.

V. References

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Appendix I

Evaluation of Dipole and Exchange Averages

It is required to evaluate the average over all orientations of the unit vectors $\mathbf{E}_i$ of expressions of the form

$$\Pi(\mathbf{E}_i \,.\, \mathbf{T}_{ij} \,.\, \mathbf{E}_j)^{n_{ij}},$$

which may be represented by geometric figures as in Table 1. The procedure differs according as the figures are *simple* (two links meet at each point, first figure in Table 1) or *knotted* (more than two links meet at one or more points, second figure in Table 1).

(*a*) *Knotted Figures*

The averages for knotted figures are reduced to sums of products of averages for simple figures by using the formulae

$$\langle(\mathbf{a}_1.\mathbf{E})(\mathbf{a}_2.\mathbf{E})\rangle = \frac{1}{3}\mathbf{a}_1.\mathbf{a}_2,$$

$$\langle(\mathbf{a}_1.\mathbf{E})(\mathbf{a}_2.\mathbf{E})(\mathbf{a}_3.\mathbf{E})(\mathbf{a}_4.\mathbf{E})\rangle = \frac{1}{15}[(\mathbf{a}_1.\mathbf{a}_2)(\mathbf{a}_3.\mathbf{a}_4)+(\mathbf{a}_1.\mathbf{a}_3)(\mathbf{a}_2.\mathbf{a}_4)+(\mathbf{a}_1.\mathbf{a}_4)(\mathbf{a}_2.\mathbf{a}_3)],$$

$$\langle(\mathbf{a}_1.\mathbf{E})(\mathbf{a}_2.\mathbf{E})\ldots.(\mathbf{a}_{2n}.\mathbf{E}) = \frac{2^n n!}{(2n+1)!}\begin{bmatrix}\text{sum of all } (2n)!/2^n n! \text{ scalars formed}\\ \text{from } \mathbf{a}_1 \ldots . \mathbf{a}_{2n} \text{ by taking scalar pro-}\\ \text{ducts of pairs}\end{bmatrix}.$$

To apply this formula to average over orientations of a dipole at a knot-point of order n (i.e. a point at which $2n$ links meet), imagine the links cut at the knot-point and joined together in pairs in all possible ways (there are $(2n)!/2^n n!$ such ways). Then the average for the knotted figure is given by

$$S = \alpha_n \Sigma' S',$$

where

$$\alpha_n = \frac{3^n 2^n n!}{(2n+1)!}.$$

Here S' means, for a given way of joining the cut links, the product of the averages for the simple figures so formed, and Σ' means summation for all ways of joining the cut links. The factor 3^n in α_n arises because the simple figures

are partially averaged ; to introduce a dipole to join each of the n pairs of cut links it is necessary to multiply by 3^n. This reduction does not depend on any property of the dyadics $\mathbf{T}_{ik}$ nor on the shape of the figures ; it is valid for dipole, exchange, or combined interactions, for any lattice, and is not restricted to nearest-neighbour interactions. If a figure contains more than one knot-point they may be reduced successively.

(b) Simple Figures

The averaging over all but one of the dipoles may be performed immediately, to give

$$S = \frac{\langle \mathbf{E}_1 \,.\, \mathbf{T}_{12} \,.\, \mathbf{T}_{23} \,.\, \ldots \,.\, \mathbf{T}_{M1} \,.\, \mathbf{E}_1 \rangle}{3^{M-1}},$$

where M is the total number of links in the figure.

In the exchange case this gives immediately

$$S_E = 1/3^{M-1}.$$

In the general case for dipole interactions the product of dyadics depends on the detailed geometry of the figure. However, for the simple cubic lattice with nearest-neighbour interactions, the angles between successive links are always 0, $\pi/2$, or π, and the dyadics may be commuted to give

$$S_D = \frac{\langle \mathbf{E}_1 \,.\, (1-3\mathbf{aa})^l \,.\, (1-3\mathbf{bb})^m \,.\, (1-3\mathbf{cc})^n \,.\, \mathbf{E}_1 \rangle}{3^{M-1}}$$

$$= \frac{\langle \mathbf{E}_1 \,.\, [1+(2^l-1)\mathbf{aa}+(2^m-1)\mathbf{bb}+(2^n-1)\mathbf{cc}] \,.\, \mathbf{E}_1 \rangle}{3^{M-1}},$$

where the unit vectors $\mathbf{a},\mathbf{b},\mathbf{c}$ are mutually orthogonal, and l,m,n are the numbers of links in the directions $\mathbf{a},\mathbf{b},\mathbf{c}$ respectively. The final averaging may now be performed, to give

$$S_D = \frac{2^l+2^m+2^n}{3^M}.$$

THE CALCULATION OF ATOMIC POLARIZATION

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Summary

By forming an equation for the potential energy of a molecule and applying the condition that this is a minimum at equilibrium, a general method of computing atomic polarizations becomes available. Expressions, in terms of bond lengths, intervalency angles, link moments, and force constants, are quoted for 10 types of polyatomic structures. The ${}_AP$'s predicted are compared with those from experiment. New measurements of the total polarizations as vapours are recorded for the species CCl_4, $SiCl_4$, CS_2, BCl_3, and $CH_3.NO_2$.

I. Introduction

Atomic polarizations, ${}_AP$, as usually determined, are highly uncertain quantities. Their calculation *a priori* is a desirable objective since the checking of observations from experiment would then be possible. The literature on the theory of the subject is sparse. Earlier workers supposed that the atomic is a fairly regular and small proportion of the electronic polarization of a substance (Sugden 1934); the measurements by Ramaswamy and Watson (1936) and particularly those by Coop and Sutton (1938) showed such a view to be erroneous. The probable additive character of atomic polarization has been noted by several authors. Audsley and Goss (1941) actually listed "atomic contributions" for carbon, hydrogen, and the halogens, but the values for the last-named were notably inconstant throughout molecules of the types RX, CX_4, and $C_6H_4X_2$. More recently, Treiber, Koren, and Schurz (1950) have proposed a procedure for obtaining the atomic polarizations of non-polar substances from their molecular refractions and certain empirical "bond constants"; however, the method fails to cover the large ${}_AP$'s established by Coop and Sutton (1938) for various classes of symmetrical molecules (e.g. for *p*-benzoquinone : ${}_AP_{\text{predicted}}=1\cdot2$ c.c., ${}_AP_{\text{found}}=8$ c.c. approx.) and must therefore be rejected.

The ascription of atom polarization to disturbances—by the electric field applied for the measurement of dielectric constant—of the vibrations of charged atomic masses about their normal relative positions in a molecular system is well known (cf. Debye 1929, p. 43); its possible deduction from spectroscopical or dispersion data has been discussed by Dennison (1926), van Vleck (1927, 1932), and others. Theoretically, ${}_AP$ can be equated to a sum of terms each containing $e^2_{\text{eff.}}/3\pi m_{\text{eff.}}\nu^2$, in which $m_{\text{eff.}}$ is the reduced mass of the nuclei vibrating along their line of centres with an infra-red frequency ν, and $e_{\text{eff.}}$ is the effective charge defined as the rate of change $d\mu/dl$ of the magnitude of the bond dipole

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moment μ with the internuclear distance l. Only simple, mainly diatomic, molecules were considered quantitatively and the ${}_AP$'s so calculated were always small.

Coop and Sutton (1938) by considering bending rather than stretching deformations, and by equating the forces of distortion with those of restoration, provided formulae giving ${}_AP$ for linear symmetric triatomic and tetra-atomic, and tetrahedral models (respectively XY_2, X_2Y_2, and XY_4); these in each instance were some multiple of $4\pi N\mu_i^2/9V$, where N is Avogadro's number, μ_i a bond polarity, and V a force constant for the bending of one link relatively to another. More recently Dekker (1945) has suggested that the atomic polarization of a bent symmetric triatomic molecule may be obtained by replacing the μ_i of the linear model by $\mu_i \sin \alpha/2$, where α is the valency bond angle at the central atom.

The argument of Coop and Sutton becomes difficult to apply to unsymmetrical structures (e.g. to XYZ or XYZ_2 forms). Accordingly a general procedure is now proposed whereby the equation for the potential energy of the molecule is formed and the condition applied that this is a minimum at equilibrium. The method has been suggested by the paper of Rollefson and Havens (1940). The case of ethylene will first be detailed as an illustration.

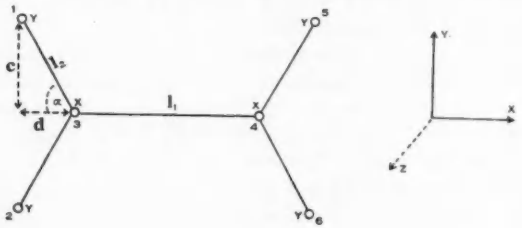

Fig. 1

II. Atomic Polarization of Ethylene*

The potential energy of ethylene, in terms of the symmetry coordinates of Herzberg's Figure 57 (H.151), is given by (1)

$$2V = \sum_{i,k=1,2,3} c_{ik}S_iS_k + c_{44}S_4^2 + \sum_{i,k=5,6} c_{ik}S_iS_k + c_{77}S_7^2 + c_{88}S_8^2 + \sum_{i,k=9,10} c_{ik}S_iS_k + \sum_{i,k=11,12} c_{ik}S_iS_k. \quad \ldots\ldots\ldots\

To rewrite (2) in terms of symmetry coordinates, the changes in the valence bonds and angles are first required. These follow from Figure 1 as

$$\beta_{12}=\frac{z_3-z_1 \text{ (or } z_2)}{l_2 \cos\alpha},$$

$$\beta_{56}=\frac{z_4-z_5 \text{ (or } z_6)}{l_2 \cos\alpha},$$

$$Q_{34}=x_4-x_3,$$

$$Q_{13}=(y_1-y_3)\sin\alpha-(x_1-x_3)\cos\alpha,$$

$$Q_{23}=-(y_2-y_3)\sin\alpha-(x_2-x_3)\cos\alpha,$$

$$Q_{54}=(y_5-y_4)\sin\alpha+(x_5-x_4)\cos\alpha,$$

$$Q_{61}=(x_6-x_4)\cos\alpha-(y_6-y_4)\sin\alpha;$$

$$\cot\alpha=\frac{d}{c},$$

$$\cot(\alpha+\delta')=\frac{d-(x_1-x_3)}{c+(y_1-y_3)},$$

$$\cot(\alpha+\delta'')=\frac{d-(x_2-x_3)}{c-(y_2-y_3)},$$

$$\cot(\alpha+\delta''')=\frac{d+x_5-x_4}{c+y_5-y_4},$$

$$\cot(\alpha+\delta'''')=\frac{d+x_6-x_4}{c-(y_6-y_4)}.$$

$$\varphi_{12}=\delta'+\delta''; \quad \varphi_{14}=-\delta'; \quad \varphi_{24}=-\delta'';$$
$$\varphi_{56}=\delta'''+\delta''''; \quad \varphi_{53}=-\delta'''; \quad \varphi_{63}=-\delta''''.$$

By inserting the values of $x_1, x_2 \ldots, y_1, y_2 \ldots, z_1, z_2 \ldots$ given by equations II 140 and II 141 of H.152, and substituting in (2), an expression for $2V$ is obtained which contains S_iS_k's, and which therefore, by comparison with (1) can yield the c_{ik}'s. Actually (1) can be shortened to

$$2V=(\text{constant})+c_{77}S_7^2+c_{99}S_9^2+2c_{9\,10}S_9S_{10}+c_{10\,10}S_{10}^2+c_{11\,11}S_{11}^2 +2c_{11\,12}S_{11}S_{12}+c_{12\,12}S_{12}^2, \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3)$$

because only infra-red active vibrations contribute to atom polarization (with ethylene, these are ν_7, ν_9, ν_{10}, ν_{11}, and ν_{12}). It follows therefore that

$$c_{77}=2k_\beta(1+\mu)^2/(l_2\cos\alpha)^2,$$

$$c_{99}=4[k_2(\sin^2\alpha+\mu)^2/\sin^2\alpha+k_\delta'(\cos^2\alpha)/l_2^2],$$

$$c_{9\,10}=-4[k_2(\sin^2\alpha+\mu)+k_\delta'(\mu+\cos^2\alpha)/l_2^2],$$

$$c_{10\,10}=4(k_2\sin^2\alpha+k_\delta'(\mu+\cos^2\alpha)^2/l_2^2\cos^2\alpha),$$

$$c_{11\,11}=4k_2(1+1/\mu)^2\cos^2\alpha+(8k_\delta+4k_\delta')(1+1/\mu)^2\sin^2\alpha/l_2^2,$$

$$c_{11\,12}=4k_2(1+1/\mu)\sin\alpha\cos\alpha-(8k_\delta+4k_\delta')(1+1/\mu)\sin\alpha\cos\alpha/l_2^2,$$

$$c_{12\,12}=4k_2\sin^2\alpha+(8k_\delta+4k_\delta')\cos^2\alpha/l_2^2.$$

Now consider ethylene subjected to an electric field E; to account for all orientations, the incident field may be taken as $E/3$ in each of the x, y, z directions. The potential energy due to $E/3$ acting along the x-direction is

$$-E(e_1x_1+e_2x_2+e_3x_3+e_4x_4+e_5x_5+e_6x_6)/3,$$

or, since $e_1=e_2=e_5=e_6=e$, and $e_3=e_4=-2e$

$$-eE(x_1+x_2+x_5+x_6-2x_3-2x_4)/3,$$

and, after inserting values for x_1, x_2 . . . from H.152,

$$-4Ee\frac{(1+1/\mu)S_{11}}{3}.$$

Similarly, the potential energies due to fields $E/3$ in the y- and z-directions are found to be

$$-4Ee\frac{(1+\mu)(S_9-S_{10})}{3},$$

and

$$-4E^e\frac{(1+\mu)S_7}{3},$$

respectively. The above three terms, each doubled, may now be added to (3), and the sum differentiated as follows:

$$\frac{\mathrm{d}(2V)}{\mathrm{d}S_7}=2c_{77}S_7-8Ee\frac{(1+\mu)}{3},$$

$$\frac{\mathrm{d}(2V)}{\mathrm{d}S_9}=2c_{99}S_9+2c_{9\,10}S_{10}-8Ee\frac{(1+\mu)}{3},$$

$$\frac{\mathrm{d}(2V)}{\mathrm{d}S_{10}}=2c_{9\,10}S_9+2c_{10\,10}S_{10}+8Ee\frac{(1+\mu)}{3},$$

$$\frac{\mathrm{d}(2V)}{\mathrm{d}S_{11}}=2c_{11\,11}S_{11}+2c_{11\,12}S_{12}-8Ee\frac{(1+1/\mu)}{3},$$

$$\frac{\mathrm{d}(2V)}{\mathrm{d}S_{12}}=2c_{11\,12}S_{11}+2c_{12\,12}S_{12}.$$

When these derivatives are equated to zero, S_7, S_9, S_{10}, and S_{11} may be evaluated from the c_{ik}'s given above.

Now the induced moment per molecule due to distortion is

$$\Sigma(ex)+\Sigma(ey)+\Sigma(ez)=4e\left(1+\frac{1}{\mu}\right)S_{11}+4e(1+\mu)(S_9-S_{10})+4e(1+\mu)S_7$$

$$=E\left[\frac{8e^2l_2^2\cos^2\alpha}{3k_\beta}+\frac{4e^2l_2^2\sin^2\alpha}{3(k_\delta'+2k_\delta)}+\frac{4e^2\cos^2\alpha}{3k_2}\right.$$

$$\left.+\frac{4e^2l_2^2\cos^2\alpha}{3k_\delta'}+\frac{4e^2\sin^2\alpha}{3k_2}\right].$$

The atomic polarization is $4\pi N/3$ times the moment caused by unit field ; noting that $el_2=\mu_{C-H}$ (the μ in the previous equations is the mass ratio used by Herzberg, H.152) we have

$$_AP=\frac{32\pi N\mu_{C-H}^2\cos^2\alpha}{9k_\beta}+\frac{16\pi N\mu_{C-H}^2\sin^2\alpha}{9(k_\delta'+2k_\delta)}+\frac{16\pi N\mu_{C-H}^2\cos^2\alpha}{9k_\delta'}+\frac{16\pi Ne^2}{9k_2}. \quad \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

III. Atomic Polarizations of Other Molecular Types

Proceeding in a similar way, atomic polarizations for the commoner types of molecular configurations have been deduced, and are written as equations (5)-(13). Stretching or bending force constants are indicated as k or k_δ respectively, the l's are bond lengths, and the e's are effective charges. Other symbols are explained by the diagrams. The $_AP$ expected from vibrations along a valency direction between two atoms is of course, $_AP=4\pi Ne^2/9k$, N is Avogadro's number.

XY_2 (Linear)

$$_AP=\frac{8\pi Ne^2}{9k}+\frac{8\pi N\mu^2}{9k_\delta}. \quad \ldots\ldots\ldots\ldots\ldots (5)$$

XY_2 (Bent)

$$_AP=\frac{4\pi N\mu^2\sin^2\alpha}{9k_\delta}+\frac{8\pi Ne^2\cos^2\alpha}{9k}+\frac{8\pi Ne^2\sin^2\alpha}{9k}\cdot\frac{p^2}{r^2}, \quad \ldots (6)$$

see Figure 2 for definitions of p, r, etc.

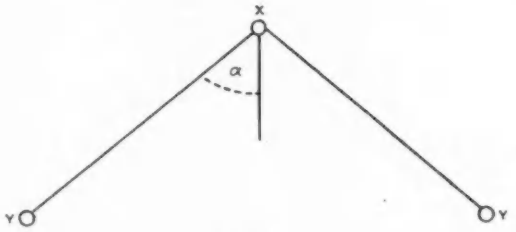

Fig. 2

$$p=1+\frac{2my}{mx}\,;\quad r=1+\frac{2my}{mx}\sin^2\alpha.$$

XYZ (Linear)

$$_AP=\frac{4\pi Ne_1^2}{9k_1}+\frac{4\pi Ne_2^2}{9k_2}+\frac{8\pi N}{9k_\delta}\left[\frac{l_2\mu_1A+l_1\mu_2B}{l_2A+l_1B}\right]^2, \quad \ldots\ldots\ldots (7)$$

see Figure 3 for definitions of A, B, etc.

X_2Y_2 (Linear)

$$_AP=\frac{8\pi Ne^2}{9k}+\frac{16\pi N\mu^2}{9k_\delta}. \quad \ldots\ldots\ldots\ldots\ldots (8)$$

X_3Y_2 (*Linear*)

$$ {}_AP = \frac{16\pi N\mu^2}{9k_\delta} + \frac{8\pi N\mu^2}{9k_{\delta'}} + \frac{8\pi Ne^2}{9k}, \quad \ldots\ldots\ldots\ldots \quad (9) $$

see Figure 4 regarding δ and δ'.

XY_3 (*Planar*)

$$ {}_AP = \frac{4\pi N\mu^2}{9k_\delta} + \frac{12\pi N\mu^2}{9k_\Delta} + \frac{12\pi Ne^2}{9k}, \quad \ldots\ldots\ldots\ldots \quad (10) $$

k_Δ refers to out-of-plane bending.

Fig. 3

$$ A = 1 + \frac{mx}{my}\left(\frac{l_1+l_2}{l_2}\right); $$

$$ B = \frac{mx}{my}\left(\frac{l_1+l_2}{l_2}\right) + \frac{mx}{mz}\,\frac{l_1}{l_2}. $$

XY_3 (*Pyramidal*)

$$ {}_AP = \frac{4\pi N\mu^2\cos^2\alpha\tan^2\beta}{9k_\delta} + \frac{12\pi Ne^2\cos^2\beta}{9k} + \frac{16\pi N\mu^2\cos^2\alpha}{9k_\delta}\left[\frac{M}{M+2m_x\cot^2\beta}\right]^2 + \frac{12\pi Ne^2}{9k}\cdot\frac{(1+\cos^2\beta)^2}{\sin^2\beta}\left[\frac{M}{M+2m_x\cot^2\beta}\right]^2, \quad \ldots. \quad (11) $$

$M = 3m_y + m_x$, β = angle between axis of symmetry and an X–Y bond, 2α = angle between two X–Y bonds, $\sin\beta = (2/\sqrt{3})\sin\alpha$.

Fig. 4

XYZ_2 (*Planar*)

$$ {}_AP = \frac{4\pi N}{9k_\Delta}\left[\frac{2\mu_1 Al_2\cos\alpha + \mu_2 l_1 B\cos\alpha}{l_2A + l_1B\cos\alpha}\right]^2 + \frac{4\pi N}{18k_{\delta'}}\left[\frac{2\mu_1 Al_2\cos\alpha + \mu_2 l_1 B\cos\alpha}{l_2A + l_1B\cos\alpha}\right]^2 + \frac{8\pi N\mu_1^2\sin^2\alpha}{9(k_{\delta'}+2k_\delta)} + \frac{4\pi Ne_2^2}{9k_2} + \frac{8\pi Ne_1^2\cos^2\alpha}{9k_1} + \frac{4\pi N}{18k_1}\left(\frac{2e_1A + e_2B}{l_2A + l_1B\cos\alpha}\right)^2 l_2^2\sin^2\alpha, $$

$\ldots\ldots\ldots\ldots\ldots\ldots$ (12)

where k_Δ refers to out-of-plane bending, k_δ refers to the change of ZYZ, k_δ' refers to the change of ZYX, see Figure 5 for definitions.

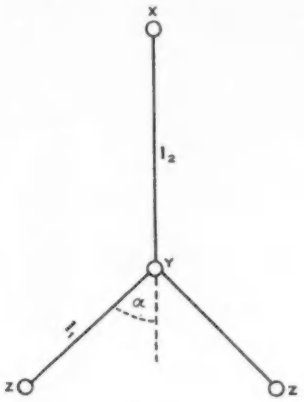

Fig. 5

$$A=1+\frac{2mz}{my}\left(\frac{l_2+l_1\cos\alpha}{l_2}\right);$$

$$B=\frac{2mz}{my}\left(\frac{l_2+l_1\cos\alpha}{l_2}\right)+\frac{2mz}{mx}\frac{l_1\cos\alpha}{l_2}.$$

XY_4 (*Tetrahedral*)

$$_4P=\frac{16\pi N\mu^2}{9k_\delta}+\frac{16\pi Ne^2}{9k}. \quad \ldots\ldots\ldots\ldots\ldots\ldots (13)$$

IV. Numerical Applications

The cases of 18 molecules are examined in Table 1. Data sources have been as follows: force constants (Herzberg 1945); interatomic distances and valency angles (Herzberg 1945; Allen and Sutton 1950); polarizations (Angyal and Le Fèvre 1952) for H_2O and H_2S; Le Fèvre, Ross, and Smythe (1950) for SO_2, Barclay and Le Fèvre (1952) for Me_2O, Ramaswamy and Watson (1936) for C_2H_2, C_2H_4, NF_3, CH_4, and CF_4, Le Fèvre and Le Fèvre (1935) for C_3O_2, Le Fèvre and Russell (1947) for NH_3 and NMe_3, Hurdis and Smyth (1943) for CH_2O, Buckingham and Le Fèvre (1953) for CMe_2O, Coop and Sutton (1938) for $SnCl_4$ and $SnBr_4$; new measurements for $MeNO_2$ and CCl_4 are reported in the present paper; where necessary electronic polarizations have been computed from refractive indexes listed in Landolt-Börnstein (1912); link moments follow, for the polar molecules, from $\mu_{\text{resultant}}$ and the appropriate valency angles, but for the non-polar molecules the values shown are assumed (cf. later).

Agreement between prediction and experiment is not good—a situation which is to be expected for a number of reasons. For instance, the quotient μ/l (which has been used for e in the calculations) is an imperfect substitute for

$d\mu/dl$ and provides an estimate of $e_{\text{eff.}}$ which is too low ; in many cases the proper value of μ_{link} is unknown (e.g. μ_{C-H} has been taken throughout Table 1 always as 0·4 D, and $\mu_{C=O}$ as the same in CH_2CO as in C_3O_2). However, undoubtedly the most likely cause of discrepancy lies with the practical measurements of ${}_AP$: for a given substance values recorded by separate workers often differ

TABLE 1

CALCULATION OF ${}_AP$ FROM μbond

	μ(bond) (D)	$10^8 l$ (cm)	2α	$10^{-5}k$	$10^{-5}k\delta/l^2$ or $10^{-5}k\delta/l_1l_2$	AP Calc. (c.c.)	AP Expt. (c.c.)
H_2O	1·51	0·958	104° 30′	7·76	0·69	0·25	0·41
H_2S	0·64	1·334	92° 16′	4·14	0·45	0·03	2·75±1·6
SO_2	1·62	1·43	120°	9·97	0·81	0·13	1·3 ±0·8
Me_2O*	1·15	1·42	111°	4·53	0·34	0·24	1·9
C_2H_2	0·4	l_{C-H} =1·06 l_{C-C} =1·207	—	5·92	0·120	0·36	1·27
C_3O_2	2·3	$l_{C=O}=l_{C=C}$ =1·16	—	15·52	0·57 ($k\delta=k\delta'$)	3·5	(c. 10)
C_2H_4	0·4	l_{C-H} =1·071 l_{C-C} =1·353	120°	5·08	0·269, $k\delta'$ = 0·567 $k\beta$ = 0·229	0·16	0·39
NF_2*	0·19	1·37	103° ($\angle\beta$ = 64° 38′)	3·5	1·0	0·01	2·09
NH_2	1·303	1·014	106° 47′ ($\angle\beta$=68° 14′)	6·32	0·61	0·78	0·45
NMe_2*	0·6	1·47	108° ($\angle\beta$=69° 8′)	8·6	0·213	0·39	?
H_2CO*	μ_{C-H} =0·4 $\mu_{C=O}$ =2·3	$l_{C=O}$ =1·21 l_{C-H} =1·09	120°	k_{CH} = 4·32 k_{CO} =10·9	$k\delta$ = 4·7† $k\delta'$ = 8·2† $k\Delta$ = 3·4†	0·15	?
Me_2CO*	μ_{C-Me} =0·4 $\mu_{C=O}$ =2·3	$l_{C=O}$ =1·14 l_{C-C} =1·57	114°	k_{CC} = 3·0 k_{CO} =10·3	$k\delta$ =20·1† $k\delta'$ =13·4† $k\Delta$ = 2·0†	0·42	2·7±1·7
$MeNO_2$*	μ_{C-N} =0·6 μ_{N-O} =3·35	l_{N-O} =1·21 l_{C-N} =1·46	130°	k_{CN} = 4·7 k_{NO} = 6·9	$k\delta$ =20·0† $k\delta'$ =16·3† $k\Delta$ = 2·1†	2·5	3·1±4·4
CH_4	0·4	1·093	—	5·04	0·461	0·11	0·08
CF_4	1·8	1·36	—	9·14	0·713	0·89	2·86
CCl_4	1·8	1·76	—	4·38	0·331	1·1	2·2
$SnCl_4$	3·0	2·30		2·80	0·075	7·83	10·3
$SnBr_4$	3·0	2·44		2·28	0·064	8·2	8·8

* The force constants of Me_2O, NF_2, NMe_2, H_2CO, Me_2CO, and $MeNO_2$ have been calculated from the frequencies listed in Landolt-Börnstein (1951) treating the methyl group as a point.

† In units of 10^{-12}.

among themselves more seriously than do those under " calc." and " expt." in Table 1. The ${}_AP$ of a molecule is found by subtraction of the electronic from the distortion polarization, and to obtain the last-named, the total polarizations plotted against $1/T$ need to be extrapolated to $1/T=0$. With polar substances this line is not horizontal, and available temperatures are such that the extrapolation necessarily always is a long one : as a result the position of the desired intercept on the P-axis is difficult to fix with certainty. In the present state of knowledge, therefore, whenever observations of polarization can be made only over a small range of temperatures, it would seem that more correct μ-values could be produced by computing ${}_DP$ as the sum of ${}_AP$ (calc.) and ${}_EP$ (usually

available from recorded refractivity data), than by attempting to fit a Debye equation : $P=A+B/T$, or by using in place of ${}_DP$ the molecular refraction for sodium light (or some constant multiple of it).

To these general considerations affecting Table 1 may be added the following particular remarks : Regarding SO_2, the standard error in the A term of the Debye equation given by Le Fèvre, Ross, and Smythe (1950) is $\pm 0{\cdot}8$ c.c., and since R_∞ cannot be claimed as definite a small ${}_AP$ remains a possibility ; similar comments are applicable to the refractivity and polarization data for H_2S and Me_2O. Regarding the trio C_2H_2, C_2H_4, and CH_4, the ${}_AP$'s run in the order found, and with the first two if higher polarities than $0{\cdot}4$ D were taken for the C—H bond prediction would become satisfactory (e.g. with acetylene the recorded

TABLE 2

CALCULATION OF LINK MOMENTS

Molecule	${}_AP$ (c.c.)	$10^8 l$ (cm)	$10^{-5}k$	$10^{-5}k_\delta/l^2$	$10^{-5}k_\triangle/l^2$	μ_{link} (D)
CO_2	0·81*	1·16	15·5	0·57	—	1·9
CS_2	1·3†	1·55	7·5	0·234	—	2·1
BF_3	2·81*	1·30	8·83	0·37	0·87	3·0
BCl_3	4·1†	1·74	4·63	0·16	0·42	3·2
SiH_4	1·79*	1·456	2·84	0·189	—	1·4
SiF_4	5·46*	1·54	7·16	0·252	—	3·1
$SiCl_4$	5·3†	2·02	3·75	0·157	—	3·1
$GeCl_4$	6·5‡	2·08	3·27	0·125	—	3·2
$TiCl_4$	3·5‡	2·21	3·11	0·100	—	2·2

* Ramaswamy and Watson (1936).

† Present paper.

‡ Coop and Sutton (1938).

${}_AP$ of 1·27 c.c. corresponds to a μ_{C-H} of 0·76 D ; this, remembering the replaceability of acetylenic hydrogen by metals, does not seem excessive). Regarding C_3O_2, it is sufficient to note that the estimate " *c.* 10 c.c." was made nearly 20 years ago from experiments on benzene solutions. Regarding NF_3, the disagreement may be due to adoption of a μ_{N-F} which is too low ; from Pauling's (1945) table of electronegativities this bond moment appears around 1 D. Regarding NMe_3 and CH_2O, although no ${}_AP$ has yet been reported for either, the practical indications are that both are small. The case of CF_4 is puzzling (since if its ${}_AP$ is 2·86 c.c. then μ_{C-F} needs to be 3·2 D) and a repetition of Ramaswamy and Watson's (1936) determination will be undertaken as soon as possible. Regarding acetone, an assumption that each Me-group contributes to the molecular ${}_AP$, the ${}_AP$ of CH_4 may explain the inadequacy of the ${}_AP$ forecast. Regarding the remaining molecules, the predicted ${}_AP$'s are reconcilable with actual observations.

For the nine molecules listed in Table 2 little information exists on the link moments involved, but since the requisite bond lengths, valency angles, and force constants are in the literature (cf. Herzberg 1945 ; Allen and Sutton 1950), equations (4)-(13) have been used in reverse to calculate μ_{link} from ${}_AP$. The

bond polarities so obtained are all higher than would be expected, following Malone (1933), from electronegativity differences. Their credibility has been briefly indicated by Le Fèvre (1953).

TABLE 3

POLARIZATIONS OF VAPOURS

Vapours	Temperature	x/x'	P (c.c.)	p (range in cm Hg)	Number of Observations	Average	$_EP$ (c.c.)	$_AP$ (c.c.)
CCl_4	343	3·848	28·35	11–18	8			
	410	3·825	28·06	17–36	6			
	415	3·802	27·92	10–35	15			
	457	3·798	27·87	12–32	15	28·05	25·89	2·2
$SiCl_4$	355	4·628	33·98	15–20	7			
	377	4·607	33·83	10–21	12			
	392	4·578	33·62	16–30	12			
	415	4·576	33·60	14–22	12	33·76	28·44	5·3
CS_2	326·5	2·961	21·74	9–25	9			
	354	2·972	21·82	15–30	12			
	387	2·923	21·46	18–30	11			
	420	2·947	21·64	18–28	11	21·66	20·39	1·3
BCl_3	294	3·296	24·19	20–30	9			
	334	3·327	24·42	13–24	18			
	362	3·273	24·03	14–24	10	24·22	20·11	4·1
				P (c.c., calc.)				
CH_3NO_2	342·5	30·92	227·1	228·5				
	361	30·00	220·3	217·5				
	384	27·83	204·3	205·4				
	397	26·92	197·6	199·2				
	416	26·20	192·3	190·8				
	428	25·36	186·2	185·9				
	433	25·20	184·9	184·0				
	443	24·40	179·2	180·1				
	465	23·42	171·9	172·2				
	487	22·49	165·1	165·2				

$P=(15\cdot2\pm4\cdot4)+(73050\pm1816)/T$

$\mu=3\cdot43\pm0\cdot04$ D.*

* Buckingham and Le Fèvre (1952) quoted 3·42 D.

V. MEASUREMENTS

As no figures existed in the literature for the polarization of silicon tetrachloride as a vapour, and because the recorded data for carbon tetrachloride (Sänger 1926), carbon disulphide (Zahn and Miles 1928; Ghosh, Mahanti,

and Mukhergee 1929; Schwingel and Williams 1930; Zahn 1930), and boron trichloride (Grassi and Puccianti 1937) did not seem altogether satisfactory, an examination has been made of these four substances (Table 3). Nitromethane has been studied for confirmatory reasons. The apparatus employed, methods, and symbols used, are explained by Le Fèvre (1953, Ch. 2).

VI. Acknowledgment

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REACTION BETWEEN A MERCURY SURFACE AND SOME FLOTATION REAGENTS: AN ELECTROCHEMICAL STUDY*

I. POLARIZATION CURVES

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[*Manuscript received September* 16, 1953]

Summary

The reactions between a mercury surface and certain flotation reagents were studied with the aid of polarization curves.

It is found that the reactions occur by electrochemical mechanisms in which oxygen plays an important part.

Cyanide ion inhibits the mercury-xanthate reaction by fixing the mercury ion concentration in equilibrium with the mercury surface.

Sodium sulphite inhibits the mercury-xanthate reaction by removing oxygen from solution.

Xanthate ion rather than xanthic acid is responsible for the formation of a mercury-xanthate film on the mercury surface.

I. INTRODUCTION

The literature reveals to date little agreement among investigators concerning the exact mechanisms involved in flotation reagent-mineral reactions. The reasons for this lack of agreement were summarized by Salamy and Nixon (1953), who investigated reactions between flotation reagents and a mercury surface hoping it would be a guide to reactions with a sulphide mineral.

Polarization curves in which the current density is plotted against the electrode potential yield considerable data concerning an electrochemical reaction. The current density is a direct measure of the rate of the reaction whilst the potential measures the metal ion concentration in equilibrium with the electrode.

Evans and Hoar (1932) first used this method of investigation to elucidate the problems of the corrosion of metals. More recently Evans (1947) summarized the experimental evidence justifying this method of attack and Petrocelli (1950) presented an excellent statement of the theory.

Any electrochemical reaction may be interpreted in terms of current flow across the metal-solution interface. The dependence of the anodic and cathodic current densities on the electrode potential, when plotted, yield polarization curves. The point of intersection of the two curves is the steady or mixed

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potential of the system, that is, the potential at which the anodic current density equals the cathodic current density.

The two reactions may be written in general as

$$M \rightarrow M^{+} + e, \qquad \text{anodic,}$$

and

$$D + e \rightarrow D', \qquad \text{cathodic.}$$

For the solutions investigated the cathodic reaction could be represented as

$$O_2 + H_2O + 4e \rightarrow 4OH',$$

whilst the anodic reaction in the case of mercury was

$$Hg + 2X' \rightarrow HgX_2 + 2e,$$

X' being an anion.

The curves may be followed away from their point of intersection by polarizing the metal with a current supplied from an external source. Under these conditions the following relationship holds

$$\begin{matrix}\text{Applied external} \\ \text{current density}\end{matrix} = \begin{pmatrix}\text{rate of} \\ \text{anodic reaction}\end{pmatrix} - \begin{pmatrix}\text{rate of} \\ \text{cathodic reaction}\end{pmatrix},$$

$$I = i_a - i_c.$$

The rate of the cathode reaction could be calculated, at a given potential, from measurements of the applied external current I, in solutions saturated with oxygen, and of the rate of the anodic reaction i_a, in solutions free from oxygen. Complete polarization curves could thereby be drawn.

It was possible to simplify the experimental technique since at any potential the drop time (and therefore the surface area) of the dropping mercury electrode was constant. Hence, at each potential, the current was proportional to the current density, so it was not necessary to measure the latter. This meant that polarographic curves, in which current was plotted against potential, sufficed to determine the various factors controlling the systems.

II. Experimental Procedure

A polarograph was constructed using a stoppered 150 ml beaker as the cell. The auxiliary electrode was a mercury pool, 2 in. in diameter, at the bottom of the beaker.

The potential of the dropping mercury electrode was measured with respect to a saturated calomel reference electrode by an electronic millivoltmeter. The liquid-liquid junction between the dropping mercury electrode and the reference calomel electrode was an agar-agar gel plug saturated with potassium nitrate.

Provision was made for removing oxygen from the solution by passing hydrogen, freed from oxygen, through a sintered glass disk. The small bubbles of hydrogen so formed enabled the oxygen to be removed from the solution within 2½ min (as shown by the magnitude of the current flowing). However, in all cases the bubbling was continued for 10 min ; the hydrogen gas escaped from the cell through a water seal.

The curve relating the applied external current and the potential for oxygen-saturated solution was determined. Oxygen was then removed and the anodic current-potential curve found. The latter curve is shown in the figures together with the cathodic current-potential curve calculated by the method of Section I.

The rate of flow of mercury through the capillary used in these experiments was 1·490 mg/sec at a potential of −1·4 V, S.C.E. At this potential the drop time was 3·76 sec.

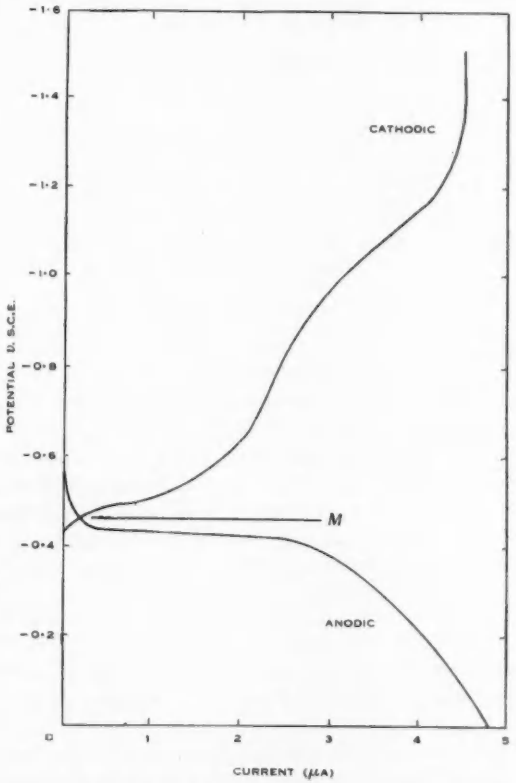

Fig. 1.—Current-potential curves for 0·1M sodium borate solution containing 0·1M potassium ethylxanthate. *M* is the mixed potential of the dropping mercury electrode in the aerated solution.

III. Mercury-Xanthate System

The current-potential curves for the anodic and cathodic reactions with mercury in the presence of various potassium ethylxanthate concentrations are shown in Figures 1–4.

It can be seen that the rate of reaction of mercury and xanthate ion was not controlled by the diffusion of either oxygen or xanthate ion to the surface when the potassium ethylxanthate concentration was greater than 0·001M. Indeed, the reaction was under mixed control and activation polarization of both the anodic and cathodic reactions was important. The anodic reaction in the region of the limiting diffusion current cannot be important from the flotation point of

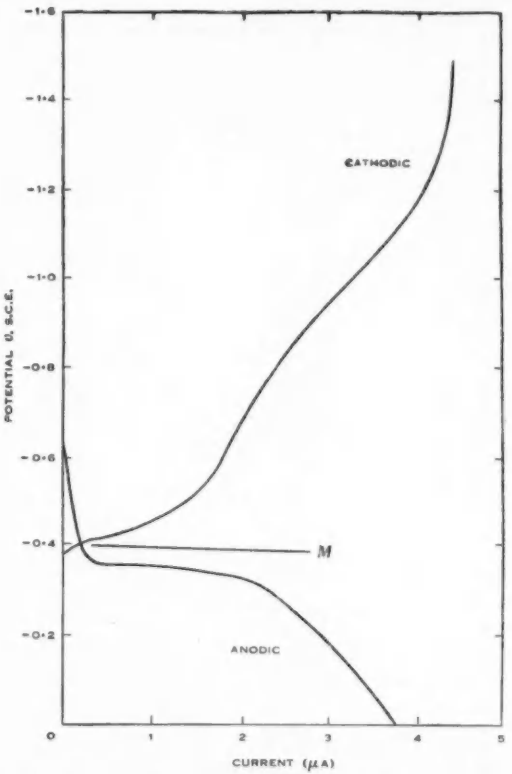

Fig. 2.—Current-potential curves for 0·1M sodium borate solution containing 0·01M potassium ethylxanthate. *M* is the mixed potential of the dropping mercury electrode in the aerated solution.

view. In this potential region the reduction of oxygen was inhibited and as a consequence the potential of the mercury electrode could never reach such a positive value if it were allowed to come to equilibrium under local action currents. Since "the limiting diffusion current" of the anodic curves was independent of concentration, the reaction in this region was under the control of activation polarization.

The rate of reaction was controlled by the diffusion of xanthate ion to the surface when the concentration of potassium ethylxanthate was 0·0001M (Fig. 4).

When the electrode was allowed to drop into the aerated solution the potential of the drop, in the later stages of its life, was *M*. It has been shown that the potential of mercury upon instantaneous immersion in the solution

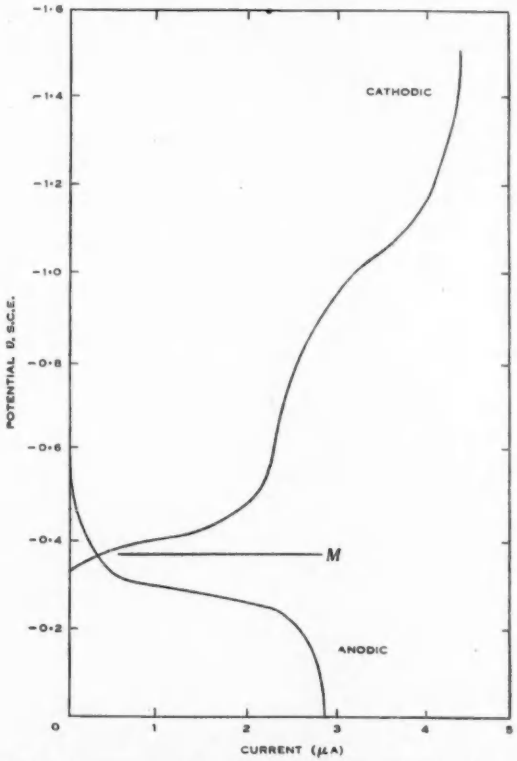

Fig. 3.—Current-potential curves for 0·1M sodium borate solution containing 0·001M potassium ethylxanthate. *M* is the mixed potential of the dropping mercury electrode in the aerated solution.

(" birth " of a droplet) will be the potential of the electrocapillary maximum (Grahame 1947). For mercury in a potassium ethylxanthate solution this potential was approximately −0·6 V, S.C.E. However, at a potential of −0·6 V, S.C.E., the rate of cathodic reduction of oxygen was high. Therefore oxygen was reduced and caused mercury to pass into solution. As a consequence, the electrode potential became more positive. This process continued until a

positive potential was reached at which the rate of cathodic reduction of oxygen became equal to the rate of the anodic dissolution of mercury.

The fact that the anodic reaction affected the cathodic reduction of oxygen at a mercury interface in a xanthate solution is consistent with the formation of a film at the mercury surface. When the film completely covered the surface the reduction of oxygen was inhibited (Salamy 1952).

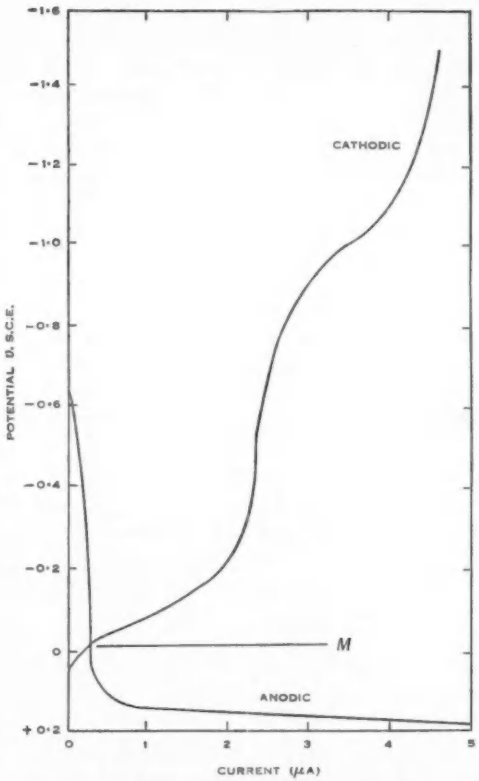

Fig. 4.—Current-potential curves for 0·1M sodium borate solution containing 0·0001M potassium ethylxanthate. *M* is the mixed potential of the dropping mercury electrode in the aerated solution.

A change in the supporting electrolyte from 0·1M sodium borate (pH 9·2) to 0·1M potassium hydroxide (pH 13) did not affect the value of the mixed potential. In addition, both the cathodic and anodic curves were unaffected at potentials more negative than −0·1 V, S.C.E. This was the potential at which mercury reacted with hydroxyl ion at a concentration of 0·1M.

It can be observed from Figures 1–4 that a tenfold change in potassium ethylxanthate concentration produced a large alteration in the value of the mixed potential. Since the pK value of ethylxanthic acid is approximately 3 (Cook and Nixon 1950), the concentration of xanthic acid present in a 0·001M

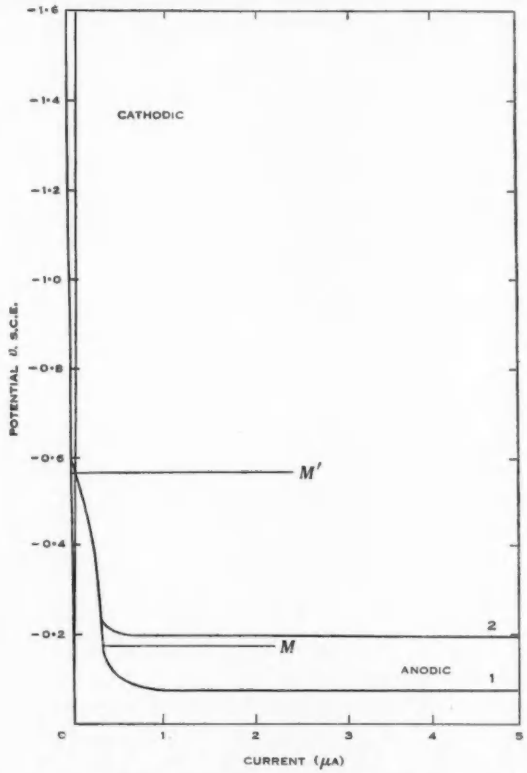

Fig. 5.—Current-potential curves for 0·1M potassium hydroxide solution containing: (i) 25 mg/l potassium *iso*amylxanthate; (ii) 25 mg/l potassium *iso*amylxanthate plus sodium sulphite. The cathodic curve shown is that associated with solution (ii); *M*, mixed potential of the dropping mercury electrode in aerated solution (i); *M'*, potential of the dropping mercury electrode in solution (ii).

potassium ethylxanthate solution at pH 9 is 10^{-3}M. Thus although the xanthic acid concentration varied by a factor of 10,000 between pH 9 and 13, the curves and the mixed potential were unaffected. The xanthate ion concentration at these two pH values was essentially constant for a given addition of potassium ethylxanthate. It is essential therefore to attribute the effect as being due to

xanthate ion, or to a combined effect of xanthate ion and xanthic acid, and not to xanthic acid alone.

It was shown by Salamy and Nixon (1953) that a hydrophobic surface was produced on a mercury surface in a solution of potassium amylxanthate. Since xanthate ions, rather than xanthic acid molecules, react with mercury it may be assumed that xanthate ions are responsible for the formation of the hydrophobic surface. This is in direct contradiction to Cook and Nixon (1950), who deduced that " neutral heteropolar molecules (free acids and free bases, neutral salts, or other neutral molecules), rather than the corresponding ions, are in general responsible for the water-repellent films that are formed on the solids that are treated with aqueous solutions of heteropolar electrolytes ".

Figure 5 presents the anodic current (curve 1) for 25 mg/l potassium amylxanthate. The mixed potential of the dropping mercury electrode in the aerated solution is indicated by *M*. (The cathodic curve is omitted from Figure 5 for the sake of clarity, but is plotted in Figure 7.)

Sodium sulphite (1·5 g) was added to a 25 mg/l potassium amylxanthate solution to remove oxygen. The anodic current (curve 2) and the cathodic current are shown in Figure 5. The mixed potential of the dropping mercury electrode was *M'*. The change in position of the mixed potential from *M* to *M'* was caused by the change in the cathodic current, as the anodic curve was only altered at the more positive potentials. It can be concluded, therefore, that the anodic reaction, the formation of the mercury xanthate reaction product, is dependent upon the presence of oxygen in solution in the absence of an applied anodic current.

IV. Mercury-Cyanide System

The current potential curves for the anodic and cathodic reactions in the presence of varying concentrations of potassium cyanide are shown in Figure 6.

During the experimental determination of these curves, it was noted that the applied potential difference between the dropping mercury electrode and the mercury pool was not proportional to the potential of the dropping mercury electrode. In some cases it was necessary to apply 0·5 V to cause the potential of the dropping electrode to become 10 mV more positive. If the usual polarographic procedure of plotting applied voltage against current had been followed the curves would have been quite incorrect. The desirability of using an independent reference electrode was thus fully illustrated.

The cathodic current was found to be independent of the cyanide ion concentration. The mixed potential of the dropping mercury electrode in each solution was the potential at which the cathodic curve 1 (Fig. 6) crossed the anodic curves 2–8. The rate of reaction of mercury in cyanide solutions more concentrated than 0·001M (curve 5) was controlled by the diffusion of oxygen to the surface. In more dilute solutions the reaction was under mixed control.

The polarization curves and the mixed potentials shown in Figure 6 apply only to the dropping mercury electrode employed and the conditions of stirring associated with it. If the polarization curves determined at a stationary electrode could be assumed to be analogous to those obtained with the dropping

mercury electrode, then the non-linear relationship between cyanide ion concentration and the experimental mixed potentials obtained by Salamy and Nixon (1952) with very dilute solutions could be explained.

There was a linear relationship between the mixed potential and the concentration of cyanide ion provided this was greater than 0·001M. At very low cyanide ion concentrations (of the order of 0·0001M) the mixed potential was

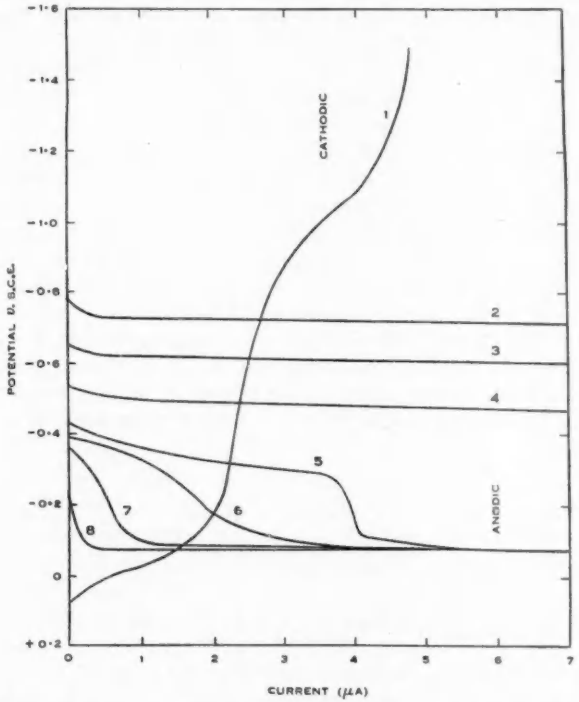

Fig. 6.—Current-potential curves of 0·1M potassium hydroxide containing various potassium cyanide additions. Curve 1, the cathodic reduction of oxygen; curve 2, anodic current for solution containing 1M potassium cyanide; curve 3, anodic current for solution containing 0·1M potassium cyanide; curve 4, anodic current for solution containing 0·01M potassium cyanide; curve 5, anodic current for solution containing 0·001M potassium cyanide; curve 6, anodic current for solution containing 0·0005M potassium cyanide; curve 7, anodic current for solution containing 0·00025M potassium cyanide; curve 8, anodic current for solution containing no potassium cyanide.

essentially that of the supporting electrolyte (0·1M potassium hydroxide). At intermediate concentrations (0·001 to 0·0001M) the mixed potential was controlled by both the cyanide ion and oxygen concentrations. In this region it will be observed that a decrease of cyanide ion concentration produced a non-linear decrease in the value of the mixed potential.

V. Mercury-Xanthate-Cyanide System

Figure 7 shows the anodic current-potential curves for 25 mg/l potassium amylxanthate (curve 1) and 25 mg/l potassium amylxanthate plus 65 mg/l potassium cyanide (curve 2). In both cases the cathodic curve was the same.

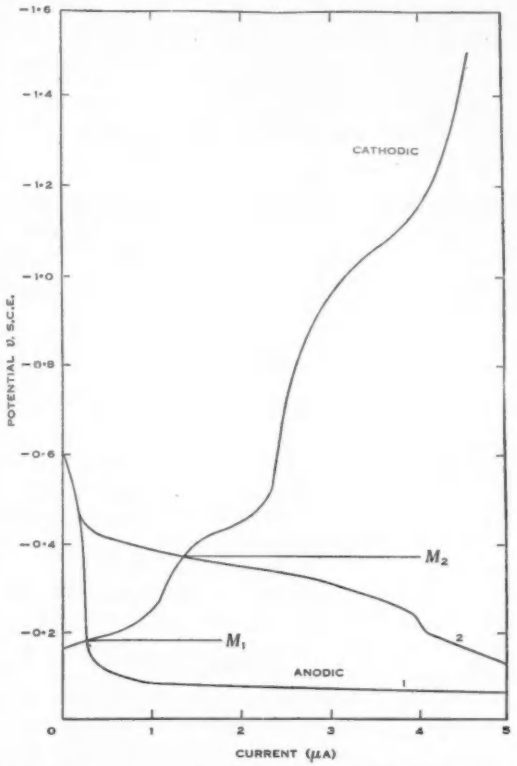

Fig. 7.—Current-potential curves for 0·1M potassium hydroxide solution containing: (i) 25 mg/l potassium *iso*amylxanthate; (ii) plus 0·001M potassium cyanide; M_1, mixed potential of dropping mercury electrode in aerated solution (i); M_2, mixed potential of dropping mercury electrode in aerated solution (ii).

The mixed potential of the dropping mercury electrode in a solution containing 25 mg/l potassium amylxanthate was M_1. The addition of potassium cyanide to the solution caused the mixed potential to change to the more negative value M_2, by limiting the mercuric ion concentration in equilibrium with the electrode. The limitation of the mercuric ion concentration was brought about by a reaction which is indicated by an alteration of the anodic current-potential curve.

VI. Conclusions

A film of xanthate at a mercury surface is formed by a mechanism which involves the electrochemical oxidation of xanthate ions and the reduction of oxygen.

Xanthate ion rather than xanthic acid is the active constituent.

If oxygen is removed from the xanthate solution as by the addition of sodium sulphite, the film-forming reaction is inhibited.

Cyanide ion inhibits the film-forming reaction by fixing the mercury ion concentration in equilibrium with the surface, at a value below that necessary for the reaction of mercury and xanthate ions.

VII. Acknowledgments

The authors gratefully acknowledge the assistance derived from stimulating discussion with Dr. T. Mills, Aeronautical Research Laboratory, Melbourne; and with Mr. G. M. Willis and Associate-Professor H. H. Dunkin, University of Melbourne. One author (S.G.S.) is indebted to the Commonwealth Research Fund for a grant which made this work possible.

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THE KINETICS OF DECARBOXYLATION OF URONIC ACIDS, POLYSACCHARIDES, AND OXYCELLULOSES

By A. MELLER*

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Summary

Measurements of the rate of carbon dioxide evolution from sulphuric acid solutions of galacturonic acid have revealed that it follows first-order kinetics, but some carbon dioxide is also formed in a side reaction at a constant rate.

Glucuronolactone, polyglucuronic acid, and oxycelluloses decarboxylate in essentially homogeneous sulphuric acid solutions according to a two-step reaction scheme, both steps being kinetically first order. This main reaction is accompanied by side reactions in which carbon dioxide is evolved at an approximately constant rate.

Mathematical expressions have been derived and applied to compute the constants of the rate equation for the decarboxylation of materials containing uronic acid.

I. INTRODUCTION

Investigations of the acid catalysed decarboxylation of uronic acids, polysaccharides, and oxycelluloses have been mainly concerned with the analytical determination of their uronic acid content. The kinetics of carbon dioxide evolution in boiling acid solutions of such materials has formed the subject of three papers (Taylor *et al.* 1947 ; Huber 1951 ; Huber and Deuel 1951). Taylor *et al.* (1947) concluded that the rate of carbon dioxide evolution from monomeric and polymeric alduronic acids, and " celluronic acids " in boiling 12 per cent. hydrochloric acid solution followed a first-order law. Huber and Deuel (1951), who were mainly interested in the influence of substituent groups in alduronic acids on the stability of the uronic acid carboxylic group, stated that the rate controlling reaction in the carbon dioxide evolution is first order. They applied experimental conditions under which the decarboxylation took place very rapidly. The rate constants derived from measurements of carbon dioxide formed at four or, in some instances, only at two reaction time periods showed in most cases a drift

In both investigations the monomeric uronic acids were decarboxylated in homogeneous solutions, but the polymeric substances may have been decarboxylated, partly, heterogeneously under the experimental conditions applied. It is probable that the physical (supermolecular or micellar) structure of polysaccharides and oxycelluloses will influence the rate of the acid catalysed decarboxylation in a heterogeneous system (Meller 1949, 1953*a*). In addition, in the interpretation of the kinetics of the carbon dioxide evolution from uronic

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acids and polysaccharides, no consideration was given to the carbon dioxide liberated from either the breakdown products formed in the acid catalysed reaction or the non-uronic acid portion of the polysaccharides used.

It was anticipated that the rate of decarboxylation of polysaccharides and oxycelluloses may be studied more conveniently by using homogeneous solutions. Polysaccharides and oxycelluloses dissolve, or disperse, in concentrated mineral acids. Such dispersions upon dilution may represent essentially homogeneous systems. It was realized that in the interpretation of the kinetics of carbon dioxide evolution from materials containing uronic acid, consideration should be given to the rates at which carbon dioxide is formed from (i) uronic acid carboxyls, (ii) breakdown products of uronic acids, and (iii) the non-uronic acid portion of these materials.

II. Experimental

(*a*) *Apparatus and Technique*

The decarboxylation apparatus used was similar to that for determining the uronic acid content of cellulosic materials described by Browning (1949), but, for maintaining a constant rate of flow of carrier gas (nitrogen), an arrangement described by Sucharda, Bobranski, and Ferguson (1936) for the semi-microdetermination of carbon and hydrogen was used. Two adsorption trains, each consisting of three tubes, were applied and a two-way cock permitted the gas to pass through one adsorption train while the second train was being weighed. The first tube of each train served to adsorb the carbon dioxide evolved; the second tube was an indicator for saturation of the adsorbent of the first tube; and the third tube acted as a counterpoise when the weight increases of the first tube were measured.

The polysaccharide and oxycellulose samples were first dispersed in 72 per cent. (w/w) sulphuric acid solution. After standing for 3–5 hr at room temperature the transparent dispersions were diluted dropwise with water with ice-cooling to solutions corresponding to either 24 per cent. (w/w) or 27 per cent. (w/w) sulphuric acid content. The reaction mixtures became turbid during dilution, but in the decarboxylation procedure they became clear after boiling for a comparatively short period of ½–1 hr.

The flask containing the test sample dispersed in the acid solution was connected to the adsorption tubes, pure nitrogen was passed through, and the apparatus filled with nitrogen was left overnight at room temperature. Check weighings showed that no carbon dioxide was evolved overnight from the acid solutions of monomeric or polymeric uronic acids. The solutions were then brought to the boil in an oil-bath while purified nitrogen was passed through the reaction flask and adsorption tubes. Zero time of reaction was taken as the visible boiling of the solutions. Some carbon dioxide was evolved before reaching the boiling temperature, but this quantity could not be determined accurately. The time needed for the carbon dioxide evolved in the reaction flask to reach the adsorption tubes was considered to compensate for the heating period during which some decarboxylation took place at a lower rate than at boiling temperature.

Monomeric uronic acids were decarboxylated in 24 per cent. (w/w) and 27 per cent. (w/w) sulphuric acid solutions with and without dispersing them first in 72 per cent. (w/w) sulphuric acid.

The decarboxylations were conducted for periods of 6–6½ hr and weighings of the carbon dioxide evolved were done at half-hourly or hourly intervals.

(*b*) *Materials*

The monomeric alduronic acids used were galacturonic acid monohydrate (Eastman Kodak Co.) and glucuronolactone (Corn Products Refining Co.). The melting point of the latter sample (171–172 °C) indicated that it may not have been pure. Alkalimetric titrations resulted in values corresponding to 100 per cent. purity for the galacturonic acid sample and 101·4 per cent. for the glucuronolactone sample. The end-point in the titration of the glucuronolactone was not well defined.

The oxycelluloses were prepared from kierboiled cotton fibres by applying 0·1N potassium dichromate in 0·2N sulphuric acid solution (Meller 1951, 1952). The chlorous acid reoxidized dichromate oxycellulose was prepared using 0·4M chlorous acid solution (Meller loc. cit.).

III. Interpretation and Discussion of Results

(*a*) *The Kinetics of Carbon Dioxide Evolution from Alduronic and Polyuronic Acids*

The rate of carbon dioxide evolution from sulphuric acid solutions of galacturonic monohydrate followed a first-order law, but carbon dioxide was also formed simultaneously by a "side reaction" at an approximately constant rate. The course of carbon dioxide evolution from galacturonic acid is expressed by the following rate equation:

$$x = a(1 - e^{-kt}) + bt, \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where x is the amount of carbon dioxide evolved at reaction time t, k the first-order rate constant, b the rate constant of the side reaction also giving carbon dioxide, and a the uronic acid content of the sample expressed in terms of carbon dioxide. The relevant results and their evaluation are presented in Table 1.

The carbon dioxide referred to as arising from the side reaction is evolved from decomposition products of uronic acids present in the sulphuric acid solutions. The molecular mechanism involved in the formation of decomposition products and of carbon dioxide from both uronic carboxyls and the decomposition products was discussed by Huber and Deuel (1951) in the light of the electronic theory of organic reactions.

A twofold amount of galacturonic acid monohydrate did not change appreciably the magnitude of the first-order rate constant as seen by the curves in Figure 1.

The rate controlling reaction of decarboxylation of polygalacturonic acid and glucuronolactone appears to take place in two reaction steps, each step

TABLE 1

DECARBOXYLATION OF MONOMERIC AND POLYMERIC GALACTURONIC ACIDS

Reaction Time (hr)	Carbon Dioxide Evolved when 24% Sulphuric Acid Solution Reacts with :			
	Galacturonic Acid Monohydrate (0·117 g)		Polygalacturonic Acid (0·297 g)	
	Expt., x (mg)	Calc., x* (mg)	Expt., x (mg)	Calc., x† (mg)
1·0	9·4	10·8	10·4	11·85
1·5	13·7	14·3	18·3	20·3
2·0	16·5	16·9	26·0	27·9
2·5	18·8	18·8	32·7	34·5
3·0	20·3	20·4	38·4	39·9
3·5	—	—	43·5	44·2
4·0	22·5	22·4	47·2	47·4
4·5	—	—	50·5	50·2
5·0	23·7	23·6	52·2	52·3
5·5	—	—	54·0	54·0
6·0	24·3	24·3	55·3	55·2
6·5	—	—	56·2	56·3

* $x = 24 \cdot 2(1 - e^{-0 \cdot 58t}) + 0 \cdot 14t$.

† $x = 57 \cdot 2\left[1 - \frac{1}{0 \cdot 54 - 1 \cdot 35}(0 \cdot 54e^{-1 \cdot 35t} - 1 \cdot 35e^{-0 \cdot 54t})\right] + 0 \cdot 30t$.

being kinetically first order. This main reaction is accompanied by a side reaction in which carbon dioxide is evolved at a constant rate.

The kinetics of the main reaction is represented by the equation for consecutive first-order irreversible reactions, namely,

$$x' = a\left[1 - \frac{1}{k_2 - k_1}(k_2 e^{-k_1 t} - k_1 e^{-k_2 t})\right]. \quad \ldots\ldots\ldots\ldots \quad (2)$$

Since carbon dioxide is also formed from the decomposition products of alduronic acids at a rate taken as constant ($x'' = k_0 t$), the total carbon dioxide formed at a reaction time t will be

$$x = a\left[1 - \frac{1}{k_2 - k_1}(k_2 e^{-k_1 t} - k_1 e^{-k_2 t})\right] + k_0 t, \quad \ldots\ldots\ldots \quad (3)$$

where a is the uronic acid carboxyl content of the test sample, k_2 the rate constant of the second step in the decarboxylation, k_1 that of the first step, and k_0 is the zero-order rate constant of the side reaction. The relevant results and their evaluation are presented in Tables 1 and 2.

On increasing the amount of glucuronolactone twofold the constants k_1 and k_2 changed very little, as seen in Figure 2.

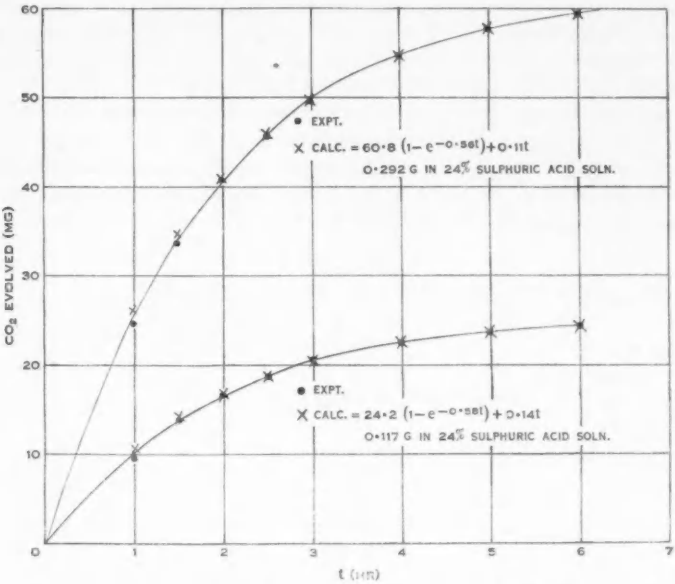

Fig. 1.—Decarboxylation of galacturonic acid monohydrate.

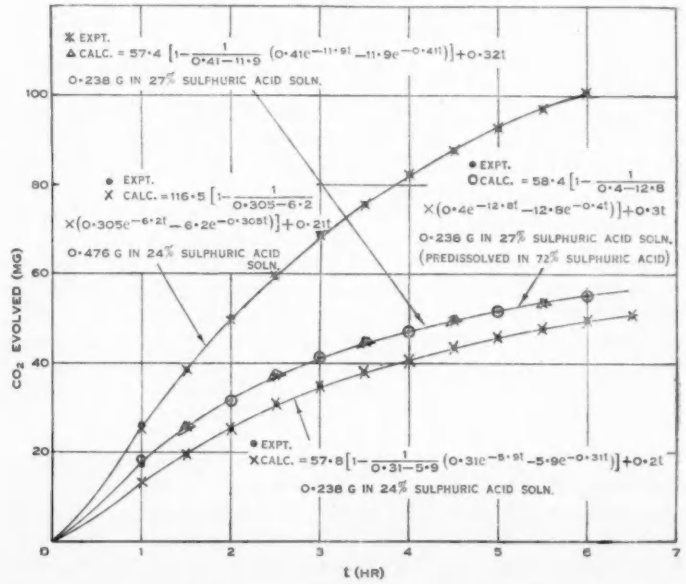
EXPT.
CALC. = 57·4 [1−1/(0·41−11·9) (0·41e^−11·9t−11·9e^−0·41t)]+0·32t
0·238 G IN 27% SULPHURIC ACID SOLN.
EXPT.
CALC. = 116·5 [1−1/(0·305−6·2) (0·305e^−6·2t−6·2e^−0·305t)]+0·21t
0·476 G IN 24% SULPHURIC ACID SOLN.
EXPT.
CALC. = 58·4 [1−1/(0·4−12·8) (0·4e^−12·8t−12·8e^−0·4t)]+0·3t
0·238 G IN 27% SULPHURIC ACID SOLN. (PREDISSOLVED IN 72% SULPHURIC ACID)
EXPT.
CALC. = 57·8 [1−1/(0·31−5·9) (0·31e^−5·9t−5·9e^−0·31t)]+0·2t
0·238 G IN 24% SULPHURIC ACID SOLN.
CO2 EVOLVED (MG)
t (HR)

Fig. 2.—Decarboxylation of glucuronolactone.

Increasing the concentration of sulphuric acid applied in the decarboxylation from 24 per cent. (w/w) to 27 per cent. (w/w) increased both rate constants, k_1 and k_2, considerably. This influence of acid concentration on the reaction mechanism has been discussed by Link and Niemann (1930), Conrad (1931), Whistler, Martin, and Harris (1940), Huber (1951), and Huber and Deuel (1951).

That the predissolving of glucuronolactone with 72 per cent. (w/w) sulphuric acid and standing overnight after diluting the reaction mixture to 27 per cent. (w/w) sulphuric acid content did not cause appreciable decarboxylation is indicated by the curves in Figure 2.

TABLE 2

DECARBOXYLATION OF GLUCURONOLACTONE AND OXYCELLULOSE

Reaction Time (hr)	Carbon Dioxide Evolved when 27% Sulphuric Acid Solution Reacts with:			
	Glucuronolactone (0·230 g)		Dichromate Oxycellulose (5·00 g)	
	Expt., x (mg)	Calc., x* (mg)	Expt., x (mg)	Calc., x† (mg)
1·0	17·3	18·3	13·7	13·6
1·5	25·6	25·7	19·2	19·2
2·0	31·7	31·9	24·2	24·0
2·5	37·0	36·95	28·2	28·2
3·0	40·9	41·2	31·7	31·9
3·5	44·4	44·5	35·1	35·15
4·0	47·2	47·4	38·1	38·0
4·5	49·6	49·8	40·6	40·6
5·0	51·8	51·7	43·0	43·2
5·5	53·5	53·4	45·2	45·2
6·0	54·8	54·8	47·2	47·3

* $x = 58{\cdot}4\left[1 - \frac{1}{0{\cdot}40 - 12{\cdot}8}(0{\cdot}40e^{-12{\cdot}8t} - 12{\cdot}8e^{-0{\cdot}40t})\right] + 0{\cdot}30t.$

† $x = 34{\cdot}5\left[1 - \frac{1}{0{\cdot}40 - 22{\cdot}9}(0{\cdot}40e^{-22{\cdot}9t} - 22{\cdot}9e^{-0{\cdot}40t})\right] + 2{\cdot}65t.$

Since the rate of decarboxylation of glucuronolactone was interpreted by a consecutive two-step reaction equation, it is possible that the slowest reaction in the first step involves a change from the lactone to the free acid, while that of the second step may constitute the actual decarboxylation of the uronic carboxyl. If this is accepted, then the rate constant of the second reaction step, k_2, should be regarded as the " characteristic rate constant of decarboxylation " of alduronic acids.

(*b*) *The Kinetics of Decarboxylation of Oxycelluloses*

The carbon dioxide evolution from dichromate and from chlorous acid reoxidized dichromate oxycelluloses appears to follow the same pattern as that of monomeric glucuronolactone. The rate controlling reaction seems to take place in two consecutive steps, both kinetically first order, upon which are

superimposed two side reactions in which carbon dioxide is formed as an approximately linear function of time. . This carbon dioxide is assumed to be formed from (i) unoxidized glucose anhydride units of the oxycelluloses and from (ii) the decomposition products of glucuronolactone units.

The rate equation for the carbon dioxide evolution from the oxycelluloses is formally the same as that for glucuronolactone, as discussed in Section III (*a*). Since the side reaction carbon dioxide arises from two sources, the zero-order rate constant will be the sum of two constants.

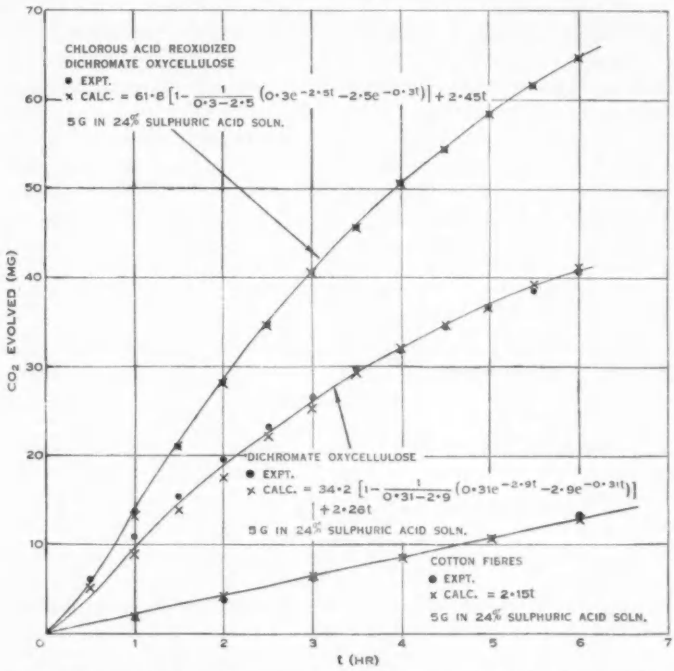

Fig. 3.—Decarboxylation of dichromate and chlorous acid reoxidized oxycelluloses, and cotton fibres.

The carbon dioxide formed at any instant is

$$x = a\left[1 - \frac{1}{k_2 - k_1}(k_2 e^{-k_1 t} - k_1 e^{-k_2 t})\right] + (k_0 + c)t, \quad \ldots\ldots\ldots (4)$$

where k_1 and k_2 are the rate constants for the two consecutive rate controlling reactions involved in the decarboxylation of the uronic acid carboxyl, k_0 and c the rate constants of the side reactions, a the uronic acid content, and t is the reaction time. The constants k_0 and c cannot be determined separately, but their sum can be evaluated. The results obtained with oxycelluloses are shown in Table 2 and by the curves in Figure 3.

It may be noted that the calculated rate constants of the first-step reaction (i.e. the k_1 values) of the oxycelluloses do not agree well with those of glucuronolactone. This may be partly due to the fact that the sulphuric acid " solutions " do not represent entirely homogeneous systems, particularly in the early stages of decarboxylation. As mentioned in Section II (*a*) the turbid dispersions of oxycelluloses in sulphuric acid turned into clear solutions after a short period of heating. In the early stages of decarboxylation hydrolysis may be entirely rate controlling, or the rate of carbon dioxide evolution may not be much different from the rate of hydrolysis of the oxycelluloses.

(*c*) *Evaluation of Rate Measurements for Determining the Rate Constants*

In computing the rate constants from the results of rate measurements some complications arise, because the initial concentrations of uronic carboxyl are not known.

If experimental values of carbon dioxide evolved (x) and time of decarboxylation (t) are known, the problem is to find the numerical values of the constants k_1, k_2, k_0+c, and of a (which is the uronic acid carboxyl content) in (**4**).

Substitution of x- and t-values in (**4**), and the customary algebraic elimination procedure is of little use for deriving the constants.

The following procedure has been developed to transform (**4**) into an approximate equation.

$$x=\underbrace{a\left[1-\frac{1}{k_2-k_1}(k_2e^{-k_1t}-k_1e^{-k_2t})\right]}_{x_1}+\underbrace{(k_0+c)t}_{C},$$

where x_1 is the uronic acid carbon dioxide, and C the side reactions carbon dioxide.

This equation can be rewritten

$$x=a-\frac{ak_2}{k_2-k_1}\left(e^{-k_1t}-\frac{k_1}{k_2}e^{-k_2t}\right)+Ct,$$

and if $k_1>k_2$, and k_1 is comparatively large, $\exp(-k_1t)$ may be neglected for higher t-values so that substitution of

$$a=\frac{ak_2}{k_2-k_1}-\frac{ak_1}{k_2-k_1},$$

yields

$$x=\frac{ak_1}{k_1-k_2}(1-e^{-k_2t})-a\frac{k_2}{k_1-k_2}+Ct,$$

which can be rewritten giving

$$x=A(1-e^{-k_2t})-B+Ct, \quad \ldots\ldots\ldots\ldots\ldots \quad (5)$$

where

$$A=\frac{a}{1-k_2/k_1},$$

and

$$B=A\frac{k_2}{k_1}.$$

To find the values for k_2, A, B, and C use has been made of the author's equation (Meller loc. cit.) by means of which these constants can be evaluated.

A second method for finding the constant k_2 was suggested by Dr. E. J. Williams, Section of Mathematical Statistics, C.S.I.R.O.

Let observations be taken at time intervals θ, and denote the corresponding carbon dioxide evolutions as x', x'', x''', and x'''', then

$$\log_e \frac{x'''' - 2x''' + x''}{x''' - 2x'' + x'} = k_2\theta.$$

Having obtained the value of the constant k_2, the other constants may be computed by simple algebraic substitution and elimination.

In all the above considerations the carbon dioxide evolved from the unoxidized portion of the oxycelluloses has been taken to be proportional to reaction time. The kinetics of carbon dioxide formation from monomeric and polymeric carbohydrates by hot acid solutions is probably much more complex in nature. However, the evolution of carbon dioxide from cotton under the experimental conditions applied in decarboxylating uronic acids, polysaccharides, and oxycelluloses is approximately linear with reaction time, as seen in Figure 3.

IV. Acknowledgments

The author is indebted to Messrs. Australian Paper Manufacturers Ltd. for permission to publish this paper; and to Miss J. F. Row and Mr. D. P. Crombie for their assistance in the experimental work.

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SOME NITRO- AND AMINO-COMPOUNDS PREPARED FROM NATURALLY OCCURRING 2,2-DIMETHYLCHROMENES

By D. J. McHugh* and S. E. Wright*

[*Manuscript received October* 26, 1953]

Summary

The preparation of some nitro-compounds of *allo*evodionol and dihydromethylevodionol is described. Aminodihydromethylevodionol has also been prepared and found to be too toxic for pharmacological use and devoid of antibiotic activity.

I. Introduction

The volatile oils of several Australian plants contain derivatives of 2,2-dimethylchromene, for example, evodionol (Lahey 1942), evodione (Wright 1948) and *allo*evodionol (Sutherland 1949). These substances were tested for pharmacological activity (Burgison, personal communication) and were found to have some vasodilator activity, but their sparing solubility in aqueous solvents makes their pharmacological study difficult. Therefore, it was decided to attempt to prepare some nitro-compounds of these substances in order to convert them to the corresponding amines, at the same time reducing the chromene nucleus to the saturated chromane.

*allo*Evodionol (I) has been shown (Sutherland 1949) to be 2,2-dimethyl-7-hydroxy-5-methoxy-8-acetylchromene. Nitration, using nitric acid in boiling ethanol, gave a bright yellow solid dinitro*allo*evodionol, m.p. 157 °C. When fuming nitric acid was used a white solid, m.p. 264 °C, possibly a nitro-dimer of *allo*evodionol was isolated. *allo*Evodionol undergoes dimerization when heated with dimethyl sulphate (Sutherland 1949), so that a nitro-dimer is not unexpected. When the nitration was carried out in ice-cold glacial acetic acid, using nitric acid, the same dimer was obtained together with a smaller amount of yellow coloured nitro*allo*evodionol, m.p. 130–131 °C. Dinitro*allo*evodionol was methylated to give a yellow solid methyldinitro*allo*evodionol, m.p. 119 °C, but attempts to reduce this compound to the diamine of the corresponding chromane with hydrogen and Raney nickel at atmospheric pressure and room temperature were unsuccessful. Bachman and Levine (1948) found that catalytic reduction of 2-methyl-3-nitrochroman with hydrogen and Raney nickel proved very difficult, requiring extreme conditions. Similar difficulty might also be expected with dinitro*allo*evodionol, since one of the nitro-groups enters the aromatic nucleus at position 6, and the other most probably at position 3, as the effect of the *p*-orientating phenolic group is likely to be transferred to position 3 in the chromene nucleus by the unsaturated linkage conjugated with the benzene nucleus.

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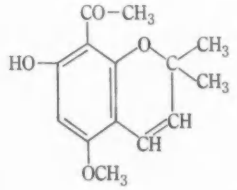
The preparation of a monoamine was then attempted, using as a starting material evodionol, 2,2-dimethyl-7-hydroxy-5-methoxy-6-acetylchromene (Lahey 1942). This compound was first reduced catalytically (Lahey 1940) to the corresponding chromane, dihydroevodionol (II, R,R′=H) which was nitrated (Lahey 1942), using nitric acid in ethanol to give 8-nitrodihydroevodionol (II, R=NO_2; R′=H). This compound was methylated with diazomethane to give 8-nitrodihydromethylevodionol, m.p. 104 °C (II, R=NO_2; R′=CH_3), which on reduction with hydrogen and Raney nickel at atmospheric pressure gave good yields of 8-aminodihydromethylevodionol (II, R=NH_2; R′=CH_3), m.p. 87 °C. This amine and its dimethyl derivative were tested for toxicity by Professor R. H. Thorp, and were found to have an LD_{50} of 200 and of 75 mg/kg respectively. These values are too high to warrant further pharmacological tests. Both compounds were also tested for antibiotic properties against several bacteria but were found to be inactive.



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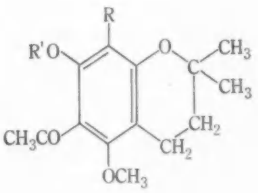

(II)

Diazotization of 8-aminodihydromethylevodionol, followed by treatment with hot saturated copper sulphate solution, was attempted in order to prepare the corresponding phenol. This phenol on methylation should give dihydroevodione (Wright 1948) but the reaction was unsuccessful, as the chromane nucleus appeared to undergo fission, giving a mixture of unidentified liquid phenols.

II. Experimental

All melting points are uncorrected. Microanalyses were carried out in the C.S.I.R.O. Microanalytical Laboratory.

(*a*) *Nitration of* allo*Evodionol.*—(i) *With Concentrated Nitric Acid.* Nitric acid (sp. gr. 1·4; 20 ml) added to *allo*evodionol (1 g) in ethanol (180 ml) was boiled gently in an open flask for 20 min. After cooling, the solution was poured into ice-cold saturated brine (250 ml), a *reddish solid* separated, which was recrystallized from ethanol in yellow needles, m.p. 157 °C. Yield 0·2 g (Found: C, 50·0; H, 4·3; N, 7·9%. Calc. for $C_{14}H_{14}O_8N_2$: C, 49·7; H, 4·1; N, 8·2%).

(ii) *With Fuming Nitric Acid.* *allo*Evodionol (1 g) in ethanol (20 ml) was treated with fuming nitric acid (2 ml), added slowly while shaking. The shaking was continued without heating until a *yellowish solid* separated; a further small amount was recovered by ether extraction of the nitration liquid. The solid was crystallized from acetone as a pale yellow solid, m.p. 264 °C (decomp.) (Found: C, 62·5; H, 5·8; N, 2·3%. Calc. for $C_{28}H_{29}O_{10}N$: C, 62·2; H, 5·5; N, 2·5%).

(iii) *With Concentrated Nitric Acid and Glacial Acetic Acid.* *allo*Evodionol (0·5 g) in a mixture of glacial acetic acid (8 ml) and acetic anhydride (1 ml) was treated with nitric acid (sp. gr. 1·4; 2 ml) at 0 °C. After 2–3 min the mixture was poured into water (50 ml) when a yellow solid separated (0·06 g) recrystallized from ethanol, m.p. 264 °C (decomp.), it was identical with the nitro-dimer described previously. The nitration mixture was extracted with ether and the ether removed after washing with bicarbonate, a small amount of dimer separated. The ether extract, on standing, deposited crystals which on recrystallization from ethanol yielded 0·02 g of a pale *yellow mononitro-compound*, m.p. 130–131 °C (Found: N, 4·5%. Calc. for $C_{14}H_{15}O_6N$: N, 4·8%).

(*b*) *Methyldinitro*allo*evodionol.*—A suspension of dinitro*allo*evodionol (0·2 g) in ether (25 ml) was treated with excess of diazomethane (prepared from nitrosomethylurea) in ether. On standing overnight all the solid dissolved. After standing a further 24 hr, the ether was removed, leaving an *orange-yellow solid*, recrystallized from ethanol, m.p. 119 °C (Found: C, 51·3; H, 4·7; N, 7·5%. Calc. for $C_{15}H_{16}O_8N_2$: C, 51·2; H, 4·5; N, 8%).

(*c*) *Nitrodihydromethylevodionol.*—Nitrodihydroevodionol (0·5 g) in ether (10 ml) was methylated overnight with excess of diazomethane. The ether was removed and a *colourless solid* obtained, recrystallized from ethanol, m.p. 104 °C (Found: C, 58·7; H, 6·1; N, 4·7%. Calc. for $C_{15}H_{19}O_6N$: C, 58·2; H, 6·2; N, 4·5%).

(*d*) *Aminodihydromethylevodionol.*—Nitrodihydromethylevodionol (0·5 g) in ethanol (30 ml) was hydrogenated in the presence of freshly prepared Raney nickel (0·5 g) for 4–6 hr at room temperature and atmospheric pressure. On removal of the ethanol, a white solid separated, recrystallized from ethanol, m.p. 86–87 °C. Yield 0·4 g (Found: C, 64·7; H, 7·8; N, 5·1%. Calc. for $C_{15}H_{21}O_4N$: C, 64·5; H, 7·5; N, 5·0%).

(*e*) *Dimethylaminodihydromethylevodionol.*—Aminodihydromethylevodionol (0·4 g) in acetone (50 ml) was methylated with dimethyl sulphate by refluxing in the presence of excess of anhydrous potassium carbonate. A white crystalline solid was isolated, recrystallized from acqueous ethanol, m.p. 62 °C (Found: C, 66·7; H, 8·2; N, 4·8%. Calc. for $C_{17}H_{25}O_4N$: C, 66·5; H, 8·2 4·6%).

III. Acknowledgments

The authors wish to thank Mr. M. D. Sutherland and Dr. F. N. Lahey for helpful advice and generous samples of *allo*evodionol and evodionol.

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STUDIES IN RELATION TO BIOSYNTHESIS

IV. ANGUSTIFOLIONOL

By A. J. Birch,* Patricia Elliott,* and A. R. Penfold†

[*Manuscript received February* 4, 1954]

Summary

Angustifolionol has been shown by its general properties and by alkaline degradation to acetone and 2,6-dihydroxy-4-methoxy-3,5-dimethylbenzoic acid (II; R″=H) to be 5-hydroxy-7-methoxy-2,6,8-trimethylbenzochromone (I; R,R′=CH_3). Its possible biogenetic relations are discussed.

I. Structure of Angustifolionol

A yellow crystalline substance, m.p. 118 °C was isolated by Penfold (1923) from the oil of *Backhousia angustifolia* Benth. by taking advantage of its insolubility in cold dilute sodium hydroxide solution. It is characterized by giving an intense bluish green colour with alcoholic ferric chloride, and it does not react with 2,4-dinitrophenylhydrazine. This substance, angustifolionol, has now been shown to have the formula $C_{13}H_{14}O_4$. Its general properties are strongly reminiscent of those of eugenin (I; R,R′=H) and eugenitin (I; R′=H, R=CH_3), insolubility in alkali being probably due chiefly to intense hydrogen-bonding between the 5-hydroxyl group and the 4-carbonyl group. Complete insolubility appears to be found only in compounds with a substituent in the 6-position as well. The resemblance is further confirmed by the similarity of the ultraviolet absorption spectra:

Eugenin (Schmid 1949)		Angustifolionol	
$\lambda_{max.}$ (mμ)	log $\varepsilon_{max.}$	$\lambda_{max.}$ (mμ)	log $\varepsilon_{max.}$
228	4·2	226	4·25
248	4·3	244	4·3
257	4·3	255	4·33
288	3·9	260	4·33
318 (inflex)	3·65	335	3·67

The infra-red spectrum possesses bands at 6·06 and 6·15μ, which would appear to correspond to a hydrogen-bonded carbonyl group. No hydroxyl band is visible, indicating that such a group if present, as indicated by the ferric chloride reaction, must take part in the hydrogen-bonding; strong bands at

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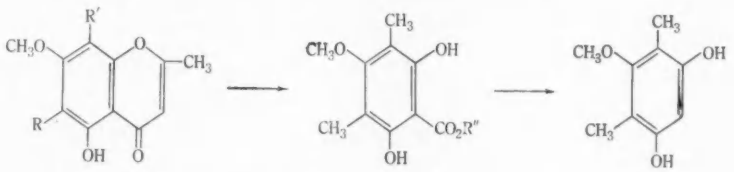
12·18 and 11·73μ may also be due to a hydrogen-bonded system. A Kuhn-Roth determination shows the presence of three *C*-CH_3 groups.

On the assumption that angustifolionol is a benzochromone derivative of type I it was hydrolysed with boiling sodium hydroxide solution under strictly defined conditions to give acetone and an acid $C_{10}H_{12}O_5$, m.p. 163 °C, methyl ester, m.p. 97 °C undepressed by authentic methyl 2,6-dihydroxy-4-methoxy-3,5-dimethylbenzoate (II ; R″=CH_3), m.p. 97–98 °C. 2,6-Dihydroxy-4-methoxy-3,5-dimethylbenzoic acid (II ; R″=H) is stated to have m.p. 156–157 °C (Herzig, Wenzel, and Graetz 1902). Brief heating converted the acid to a phenol $C_9H_{12}O_3.H_2O$, m.p. 149–150 °C undepressed by authentic 5-hydroxy-3-methoxy-2,4-dimethylphenol monohydrate (III). The synthesis of these compounds is described in Section III. The phenol from the degradation gave no colour with 2,6-dichloroquinone chloroimide at pH 9, thus confirming the presence of substituents *para-* to the hydroxyl groups.

From this evidence there is no doubt that angustifolionol is (I ; R,R′=CH_3).



(I ; R,R′=CH_3) (II ; R″=H) (III)

II. Biogenetic Relationships

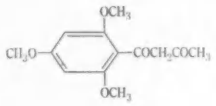
The compounds (I ; R,R′=H, I ; R=CH_3, R′=H), the *O*-desmethyl derivative corresponding to (I ; R=H, R′=CH_3), and eugenone (IV) occur together in wild *Eugenia caryophyllata* (L.) Thunb. The discovery of angustifolionol (I ; R,R′=CH_3), completing the series of all possible degrees of methylation of the phloroglucinol ring, lends further support to the hypothesis that the methyl groups in such compounds are introduced after the formation of the main skeleton. The main skeleton of the molecule could be derived by the head-to-tail linkage of five acetate units (Birch and Donovan 1953) as in V.

In view of the ready occurrence of both *O*- and *C*-methylation in phloroglucinol derivatives a similar close biogenetic relationship may exist between many other methylated phloroglucinol derivatives, particularly those containing *C*-acyl groups. Three examples among many may be quoted : aspidinol (VI),* baeckeol (VII), and xanthoxylin (VIII). The methylene bisacylphloroglucinols from *Filix mas* (cf. Birch 1951 ; Birch and Todd 1952) are further examples : flavaspidic acid (IX)* contains both *O*-CH_3 and *C*-CH_3 in otherwise identical portions of the molecule.

* These compounds are enolic, but because of lack of evidence on the direction of enolization the keto-forms are shown.

Other groups which may have been introduced in a similar manner contain isoprenoid nuclei ; from among numerous examples may be quoted evodionol (X) and euparin (XI) and a number of flavones, *iso*flavones, and coumarins.

There is no evidence to show whether the *C*-alkylation might be expected to occur after cyclization or in the open-chain β-polyketone corresponding to V. When an oxygen is missing from the ring, for example, in XI, angustione (XII), or XIII (R=CH_3, or CH=CHCH=$CHCH_3$) (Kuhn and Staab 1953), the latter



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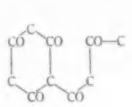

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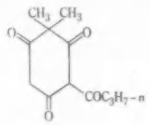

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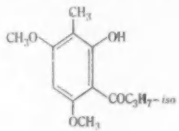

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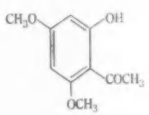

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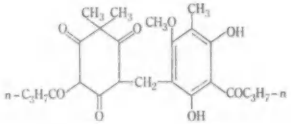

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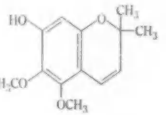

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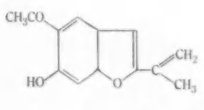

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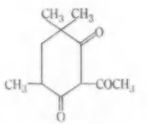

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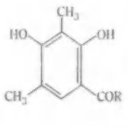

(XIII)

route seems more probable since the anionoid activity of a resorcinol group is much reduced. It is unlikely that an oxygen would be removed directly from an aromatic ring ; reduction at an open-chain stage seems much more probable (cf. Birch and Donovan 1953). Methylation of an oxygen could occur at any stage ; in eugenone (IV) it would necessarily occur after the ring closure which would generate a free phenolic group.

III. Experimental

The isolation of angustifolionol was carried out as described by Penfold (1923). The substance formed pale yellow prisms from ethanol, m.p. 118 °C (Found : C, 66·7 ; H, 6·0 ; OCH_3, 13·4 ; *C*–CH_3, 17·0%. Calc. for $C_{13}H_{14}O_4$: C, 66·7 ; H, 6·0 ; $1\times OCH_3$, 13·2 ; $3\times$*C*–CH_3, 19·2%). Reduction with magnesium and hydrochloric acid in methanol gave a bright yellow solution (flavylium salt) indicative of a chromone ring. No reaction was observed with 2,4-dinitrophenylhydrazine.

(*a*) *Hydrolysis of Angustifolionol.*—Angustifolionol (150 mg) in warm ethanol (5 c.c.) was added to a boiling solution of potassium hydroxide (3% ; 20 c.c.). The solution was rapidly distilled for 5 min, about 7 c.c. of distillate being collected. The distillate when added to 2,4-dinitrophenylhydrazine in 2*N* hydrochloric acid gave the acetone derivative (84 mg), m.p.

123–125 °C undepressed by an authentic specimen. Acidification of the residual solution gave a crystalline solid, which was taken up by extraction with ether (2 × 25 c.c.), and then passed from the ether into potassium bicarbonate solution. The reprecipitated 2,6-dihydroxy-4-methoxy-3,5-dimethylbenzoic acid (85 mg) had m.p. 163 °C and owing to its instability was not recrystallized (Found: C, 55·8; H, 5·8%. Calc. for $C_{10}H_{12}O_5$: C, 56·6; H, 5·7%). Treatment with ethereal diazomethane for 15 min gave methyl 2,6-dihydroxy-4-methoxy-3,5-dimethylbenzoate, which crystallized from aqueous methanol as needles, m.p. 97 °C undepressed by an authentic specimen (see below), m.p. 97–98 °C.

The acid was heated to 160 °C for 2 min with evolution of carbon dioxide to produce 5-hydroxy-3-methoxy-2,4-dimethylphenol, which crystallized first from acetone-light petroleum (b.p. 40–60 °C) in the presence of a trace of water, and then from water, as massive colourless prisms, m.p. 149–150 °C undepressed by an authentic specimen (see below), m.p. 148–149 °C (Found: C, 57·8; H, 7·7%. Calc. for $C_9H_{12}O_3.H_2O$: C, 58·0; H, 7·5%). It may be noted that several similar compounds contain water of crystallization. The substance was soluble in sodium hydroxide solution, but not in bicarbonate; at pH 9 in a phosphate buffer it gave with 2,6-dichloroquinone chloroimide in acetone only a pink colour, which slowly deepened to brown. It gave no colour with alcoholic ferric chloride, and did not react with 2,4-dinitrophenylhydrazine.

(*b*) *5 - Hydroxy - 3 - methoxy - 2,4 - dimethylphenol* (*III*). — Methyl - 2,4,6 - trihydroxy - 3,5-dimethylbenzoate (Robertson and Whalley 1951) (1 mole) in ether containing a trace of methanol was added to ethereal diazomethane (1 mole) and left for 3 hr. Evaporation of the solvent and crystallization from aqueous methanol gave methyl-2,6-dihydroxy-4-methoxy-3,5-dimethylbenzoate, m.p. 97–98 °C (Found: C, 58·3; H, 6·3%. Calc. for $C_{11}H_{14}O_5$: C, 58·4; H, 6·2%). The ester was refluxed with potassium hydroxide solution (10%) for 5½ hr under nitrogen and the solution acidified and extracted with ethyl acetate (4 × 10 c.c.). After evaporation of the solvent the 1,5-dihydroxy-3-methoxy-2,4-dimethylbenzene was crystallized from water as prisms, m.p. 148–149 °C undepressed by the substance obtained from angustifolionol.

IV. Acknowledgments

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THE PREPARATION OF SOME DIPEPTIDES CONTAINING ASPARAGINE, ASPARTIC ACID, AND GLUTAMINE

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Summary

Several methods have been used to synthesize dipeptides of asparagine, glutamine, and aspartic acid. Not all techniques were found to be equally applicable to the synthesis of a particular peptide. The methods examined involved the use of the chloracetyl-, phthalyl-, and carbobenzoxy- residues as protective groups and the use of acid chlorides, mixed carbonic anhydrides, phosphite amides, and *iso*cyanate esters in the coupling reaction.

I. Introduction

The main object of the present work was the preparation of peptides of asparagine and glutamine to be used in experiments on the kinetics of hydrolysis of the amide bond. Some of the results of this kinetic work have already been published (Leach and Lindley 1953*a*, 1953*b*). During the course of this work experience of a number of techniques of peptide synthesis has been acquired and it is considered that some general survey may be of interest to other workers in this field.

II. Discussion

(*a*) *The Nature of the Protecting Group*

Three types of protecting group have been used in the present work: chloracetyl, phthalyl, and carbobenzoxy groups. The chloracetyl group is only of use for the synthesis of glycyl peptides. For this purpose it is eminently satisfactory, but the chloracetyl chloride used should be freshly distilled or difficulties may be experienced in the crystallization of the derivatives. The phthalyl group has been increasingly mentioned in the literature in recent years (e.g. Billman and Hartung 1948; Sheehan and Frank 1949; Boissonas 1951). Phthalylamino acids are readily prepared by fusion of the amino acid with phthallic anhydride and are readily crystallizable solids of higher melting point than the corresponding carbobenzoxy derivatives. Removal of the phthalyl group is brought about by refluxing with alcoholic hydrazine or phenylhydrazine. Three disadvantages of the phthalyl method may be noted:

(i) For some amino acids the formation of the phthalyl derivative is accompanied by racemization (King and Kidd 1949), although conditions may usually be chosen under which this does not occur (Sheehan, Chapman, and Roth 1952).

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(ii) Many phthalylamino acids and peptides are very readily converted by dilute alkali to the corresponding *o*-carboxybenzoyl derivatives. This group cannot then be removed by hydrazine treatment. From a practical standpoint this means that all methods of peptide synthesis leading to the production of the phthalyl peptide ester cannot be used in conjunction with low temperature saponification of the ester group by alkali since this also causes opening of the phthalyl ring. Acid hydrolysis of the ester group has been used but the conditions employed would almost certainly have caused hydrolysis of the amide group of our peptides (cf. Leach and Lindley 1953*a*, 1953*b*) and hence we have not investigated this technique. Moreover, it is also impossible to prepare free peptide esters from their phthalyl derivatives since treatment with hydrazine to remove the phthalyl group also converts the ester to a hydrazide.

(iii) A final disadvantage we have noted in the present work is that the phthalyl group may occasionally prove very difficult to remove. Thus we failed entirely to remove the protecting group from phthalyl derivatives of glycyl-glycyl-L-asparagine and glycyl-DL-phenylalanyl-L-asparagine by hydrazine treatment. The reason for this is not known. No difficulty is experienced, for example, with phthalylglycylglycine or phthalylglycylasparagine and Schumann and Boissonnas (1952) claim to have successfully dephthalylated peptides of greater chain length.

Literature on the carbobenzoxy group is so extensive that little comment is necessary on its use. A practical point worthy of mention, however, is that the removal of the carbobenzoxy group from peptides containing leucine may present difficulties. We believe that this is due to the impure nature of many commercial samples of leucine which are often contaminated with methionine. The presence of any sulphur compound leads to rapid poisoning of the palladium catalyst used for removal of the carbobenzoxy group by catalytic hydrogenation.

(*b*) *The Nature of the Coupling Reaction*

Four main types of coupling reaction have been studied in some detail in the course of the present work. They are : the use of acid chlorides, the mixed carbonic anhydride technique, the chlorophosphite method, and the use of *iso*cyanate esters. The methods will be considered separately in this order.

Apart from the specialized use of chloracetyl chloride referred to earlier, the investigation of the use of acid chlorides in the present work has been confined to the reaction between phthalylamino acid chlorides and salts of amino acids in weakly alkaline buffered solution. Two groups of workers claim to have used this method successfully. Thus Grassman and Schültz-Uebbing (1950) coupled phthalylamino acid chlorides in dioxane with the aqueous solutions of sodium salts of amino acids containing an equivalent of sodium bicarbonate. Sheehan and Frank (1949) employed a similar procedure but used magnesium oxide as the alkali. In our hands, despite numerous attempts, the method proved almost completely unsuccessful. On one occasion phthalylglycylglycine was prepared using Sheehan and Frank's conditions. Repetition of this and attempts to extend it to other peptides were uniformly unsuccessful, the only products which could be isolated being the unchanged amino acid and the phthalylamino

acid corresponding to the original acid chloride. It would thus appear that phthalylamino acid chlorides react preferentially with water under these conditions. In view of the points mentioned earlier no attempt was made to study the interaction of these acid chlorides with amino acid esters.

Boissonnas (1951), Vaughan (1952), and Vaughan and Osato (1952) have described methods in which the phthalyl or carbobenzoxyamino acid is treated with one equivalent each of a tertiary base and an alkyl chlorocarbonate in a suitable dry organic solvent. This leads to the formation of a mixed anhydride of the acylamino acid and alkylcarbonic acid together with the base hydrochloride. In our work we have used exclusively triethylamine and ethyl chlorocarbonate as these were most readily available. These mixed carbonic anhydrides will react with either an aqueous solution of the sodium salt of a second amino acid or an organic solution of an amino acid ester to give the corresponding peptide derivative, carbon dioxide and ethanol being produced as by-products. Despite the lower yields obtained by reaction with the sodium salts in aqueous solution we have preferred this technique in our work to reaction with the amino acid ester in order to avoid the subsequent saponification step with its attendant risk of deamidation of asparagine and glutamine peptides. The method proved satisfactory for the preparation of peptides of the type glycyl- and leucylasparagine, yields being of the order of 50 per cent. at the stage of the acyl dipeptide. In our hands this method failed completely when attempts were made to prepare asparaginyl dipeptides. Low yields and the difficulty of separation also made the method unsuitable for the preparation of peptides of the type acylglycylaspartic acid. Coupling with the ester (preferably benzyl) of aspartic acid was more satisfactory in this case.

The most satisfactory technique for the preparation of asparaginyl dipeptides was that involving the phosphite amide. The preparation of asparaginylglycine using diethyl chlorophosphite (Anderson, Welcher, and Young 1951) has been described by Miller and Waelsch (1952). A more satisfactory reagent, however, is *o*-phenylene chlorophosphite (Anderson *et al.* 1952). This is easier to prepare and far more stable than the diethyl compound. On distillation even *in vacuo*, diethyl chlorophosphite is liable to decompose suddenly giving an orange powder and liberating a spontaneously inflammable gaseous by-product. On the other hand, we experienced no difficulty in fractionating the *o*-phenylene derivative. A practical point of some importance in this procedure is the purity of the toluene used as solvent in the coupling procedure, and removal of thiophene impurities by preliminary treatment with concentrated sulphuric acid brings about a marked improvement in yield.

Goldschmidt and Wick (1952) have published an attractive method in which the acylamino acid is condensed with an amino acid ester *iso*cyanate to give the acyl peptide ester directly, carbon dioxide being the only by-product. Carbobenzoxyasparaginylglycine ethyl ester was prepared in this way although in much lower yield than those given by Goldschmidt and Wick for simpler peptides. Absolutely anhydrous conditions are essential: even with all precautions, in the case of carbobenzoxyasparagine and glycine ethyl ester *iso*cyanate a compound which was almost certainly biscarbethoxymethylurea

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was always isolated. It seems possible therefore that a condensation reaction possibly involving two molecules of carbobenzoxyasparagine with elimination of a molecule of water may be occurring. The two reactions can apparently be formulated as

$$\text{(i)}\ \mathrm{RNCO + R'COOH \rightarrow R{\cdot}NH{\cdot}CO{\cdot}R' + CO_2},$$

$$\text{(ii)}\ \mathrm{2RNCO + 2R'COOH \rightarrow CO_2 + (R'CO)_2O + (RNH)_2CO}.$$

Fry (1953) has recently made a study of the reaction between carboxylic acids and *iso*cyanates and finds that a mixture of products corresponding to both reaction paths is usually found. No systematic search was made for the linear anhydride of carbobenzoxyasparagine in this case, but a compound was isolated on one occasion from an attempted coupling by the mixed carbonic anhydride technique of carbobenzoxyasparagine and glycine ethyl ester which had an analysis corresponding to a dimer of carbobenzoxyasparagine minus one molecule of water. No further attempt was made to characterize this compound, but its ready formation may explain the anomalous behaviour of carbobenzoxyasparagine in coupling reactions.

III. Experimental

Melting points are uncorrected. Analyses are by Dr. K. W. Zimmerman, C.S.I.R.O. Microanalytical Laboratory.

(*a*) *Phthalylglycyl-*L*-asparagine.*—To phthalylglycine (0·05 mole) in 100 ml dry dioxane at 5 °C was added triethylamine (0·05 mole) and then redistilled ethyl chlorocarbonate (0·05 mole) with stirring. After standing for 5 min, L-asparagine (0·05 mole) in NaOH (1N ; 50 ml) was added rapidly with shaking. A vigorous evolution of carbon dioxide occurred. Acidification with 1N hydrochloric acid yielded 79% of the theoretical quantity of crude product, m.p. 194–198 °C. Two extractions with hot dioxane left a 72% yield of product, m.p. 202–203 °C. Recrystallization from water gave needles, m.p. 205–207 °C (Found : C, 52·9 ; H, 4·2 ; N, 12·6%. Calc. for $C_{14}H_{13}O_6N_3$: C, 52·8 ; H, 4·1 ; N, 13·2%).

(*b*) *Carbobenzoxyglycyl-*L*-asparagine.*—This was prepared similarly and had m.p. 129·5–131 °C (Found : C, 51·8 ; H, 5·5 ; N, 12·7%. Calc. for $C_{14}H_{17}O_6N_3$: C, 52·0 ; H, 5·3 ; N, 13·0%).

(*c*) *Glycyl-*L*-asparagine.*—Prepared by three methods : (i) By reaction of chloracetyl-L-asparagine (3 g) with 0·88 ammonia (30 ml) for 3 days at room temperature, followed by evaporation to dryness, and recrystallization from ethanol-water.

(ii) By reacting phthalylglycyl-L-asparagine (0·01 mole) with hydrazine (1M ; 10 ml) in ethanol (30 ml) under reflux for 2 hr, followed by treatment with HCl (2N ; 25 ml) at 40 °C for 10 min. The peptide hydrochloride was retained on passing through a column of " Amberlite 1R–4 " in the basic form, and was displaced with 1N HCl.

(iii) By reducing carbobenzoxyglycyl-L-asparagine in 90% ethanol-water solution with Pd-H_2. The product had m.p. 220 °C. Paper chromatography using phenol as solvent gave only one brown peptide spot of R_F value 0·58 (identical with threonine). Potentiometric titration with 1·0N NaOH suggested a purity of 95% (Found : C, 38·4 ; H, 5·9 ; N, 21·4%. Calc. for $C_6H_{11}O_4N_3$: C, 38·1 ; H, 5·8 ; N, 22·2%).

(*d*) *Phthalyl-*DL*-alanyl-*L*-asparagine.*—Prepared as for (*a*), m.p. 197·5–199 °C. Yield 50% (Found : C, 54·1 ; H, 4·7 ; N, 11·8%. Calc. for $C_{15}H_{15}O_6N_3$: C, 54·0 ; H, 4·5 ; N, 12·6%).

(*e*) *Phthalyl-*L*-alanyl-*L*-asparagine.*—Prepared as for (*a*), m.p. 201–202 °C (Found : C, 54·0 ; H, 4·9 ; N, 12·2%. Calc. for $C_{15}H_{15}O_6N_3$: C, 54·0 ; H, 4·5 ; N, 12·6%).

(*f*) *Phthalyl-*L*-leucyl-*L*-asparagine.*—Prepared as for (*a*), recrystallized from dioxane/water and water. Yield 65%, m.p. 210–212 °C (Found: C, 58·3; H, 5·8; N, 10·8%. Calc. for $C_{16}H_{21}O_6N_3$: C, 57·5; H, 5·6; N, 11·2%).

(*g*) *Carbobenzoxy-*L*-leucyl-*L*-asparagine.*—Prepared as for (*b*) in 55% yield, m.p. 162–163 °C, recrystallized from boiling water after ethyl acetate extraction (Found: C, 56·9; H, 6·7; N, 11·2%. Calc. for $C_{16}H_{25}O_6N_3$: C, 57; H, 6·6; N, 11·1%).

(*h*) L-*Leucyl-*L*-asparagine.*—Prepared by hydrogenation of (*g*) as in (*c*) (iii) and recrystallized from ethanol-water, m.p. 219–222 °C. R_F value of 0·85 in phenol (identical with leucine). Potentiometric titration with 1·0N NaOH suggested a purity of 99% (Found: C, 49·4; H, 7·7; N, 17·2%. Calc. for $C_{10}H_{19}O_4N_3$: C, 48·9; H, 7·7; N, 17·2%).

(*i*) *Glycyl-*DL*-glutamine.*—Made from 2·0 g of chloracetyl-DL-glutamine (Thierfelder and von Cramm 1919) giving a 50% yield of peptide, m.p. 205 °C. Potentiometric titration suggested a purity of 86·6% (Found: C, 41·1; H, 6·8; N, 19·8%. Calc. for $C_7H_{13}O_4N_3$: C, 41·4; H, 6·4; N, 20·7%).

(*j*) *Phthalyl-*L*-leucyl-*L*-glutamine.*—Made as for (*a*). The product was extracted with chloroform to remove unreacted phthalyl-L-leucine, and was recrystallized from ethyl acetate, m.p. 123–126 °C. Yield 30% (Found: C, 58·0; H, 6·1; N, 10·8%. Calc. for $C_{19}H_{23}O_6N_3$: C, 58·6; H, 5·9; N, 10·8%).

(*k*) L-*Leucyl-*L*-glutamine.*—Prepared from (*j*) by dephthalylation as in (*c*) (ii) in 34% yield, m.p. 227–229 °C. Chromatography showed the presence of only one ninhydrin-reacting substance of R_F value 0·85 in phenol-water. Potentiometric titration with 1·0N NaOH gave a purity of 93·2% (Found: C, 50·2; H, 8·0; N, 16·1%. Calc. for $C_{11}H_{21}O_4N_3$: C, 51·0; H, 8·1; N, 16·2%).

(*l*) *Carbobenzoxy-S-benzyl-*L*-cysteinyl-*L*-asparagine.*—Prepared from carbobenzoxy-*S*-benzyl-L-cysteine (Harington and Mead 1936) and L-asparagine by the ethyl chloroformate procedure as in (*a*). Recrystallized from methanol, m.p. 180–181 °C (Found: N, 8·9; S, 7·1%. Calc. for $C_{22}H_{25}O_6N_3S$: N, 9·1; S, 7·0%).

(*m*) L-*Cysteinyl-*L*-asparagine.*—Prepared from (*l*) by reduction with sodium in liquid ammonia. The peptide was separated first as the mercury complex (Harington and Pitt-Rivers 1944) and finally as the cuprous mercaptide using cuprous oxide. In this procedure, the solution was warmed to 40–50 °C and fresh cuprous oxide added to a faint pink colour in the precipitate. This was centrifuged, washed, and decomposed with H_2S, crystallized from ethanol-water, m.p. 250 °C (Found: N, 16·4; S, 13·5%. Calc. for $C_7H_{13}O_4N_3S$: N, 17·9; S, 13·6%). Titration with iodine gave 97·4% of theoretical value. Potentiometric titration with 1·0N NaOH gave 98·7% of theoretical value.

(*n*) *Carbobenzoxy-*L*-asparaginylglycine Ethyl Ester.*—(i) Prepared from the corresponding α-aspartyl compound (9·2 g; made by Le Quesne and Young's (1952) procedure in which carbobenzoxyaspartic anhydride is condensed with glycine ethyl ester and the α- and β-aspartyl compounds are separated in a crystalline form by 30 successive extractions with sodium bicarbonate followed by acidification). This was treated with PCl_5, followed by ethereal ammonia, and the product recrystallized from hot water, m.p. 184–185 °C. Yield 4·1 g (44%) (Found: C, 54·8; H, 6·0; N, 11·8%. Calc. for $C_{16}H_{21}O_6N_3$: C, 54·7; H, 6·0; N, 12·0%).

(ii) By the chlorophosphite method (Miller and Waelsch 1952*a*). For this preparation, diethyl chlorophosphite was allowed to react with glycine ethyl ester (8·6 g) in the presence of triethylamine. The product was then refluxed in toluene (200 ml) with carbobenzoxyasparagine (21 g) giving 7 g (24%) of protected dipeptide ester after recrystallization from boiling water, m.p. 181–183 °C.

(iii) By the ester *iso*cyanate method (Goldschmidt and Wick 1952), carbobenzoxyasparagine (10·5 g) and glycine ester *iso*cyanate (5·1 g) were warmed to 90 °C in dry dioxane until CO_2 evolution ceased. Crystallization was completed by adding ether. The product was filtered off, dissolved in hot water, and sodium bicarbonate added to pH 7·5. The solution was filtered hot and on cooling a 30% yield of needle-shaped crystals, m.p. 180–182 °C was obtained.

Acidification of the mother liquor caused crystallization of a 15% yield of the symmetrical urea, m.p. 144–145 °C which was raised to 147 °C by recrystallization from ethanol and subsequently from water. Fischer (1901) gives m.p. 146 °C (Found: N, 11·8%. Calc. for biscarbethoxymethylurea ($C_9H_{16}O_5N_2$): N, 12·1%). Potentiometric titration showed that the product had no titratable carboxyl or amino-groups.

(*o*) *Carbobenzoxy-*L*-asparaginylglycine.*—Prepared by saponification of 10 g of (*n*) in methanol (500 ml) with NaOH (1·1 equiv.) for 10 min at 40 °C. Some deamidation appeared to occur and the best product which could be obtained had m.p. 166–169 °C after recrystallization from hot water. The yield was 5 g (54%) (Found: C, 51·6; H, 5·3; N, 12·0%. Calc. for $C_{14}H_{17}O_6N_3$: C, 52·0; H, 5·3; N, 13·0%).

(*p*) *L-Asparaginylglycine.*—Carbobenzoxy-L-asparaginylglycine (4 g) was reduced with hydrogen in the presence of palladium. The product was recrystallized from water giving a yield of 1·8 g (75%), m.p. 207–208 °C. The R_F value was 0·14 in collidine and only one " spot " was observed. Potentiometric titration with 1·0N NaOH gave a purity of 86·7% (Found: C, 38·5; H, 6·1; N, 21·0%. Calc. for $C_6H_{11}O_4N_3$: C, 38·1; H, 5·9; N, 22·2%).

(*q*) *Carbobenzoxy-*L*-aspartyl-*S*-benzyl-*L*-cysteine Ethyl Ester.*—Carbobenzoxyaspartic acid (11 g) was treated with cold acetic anhydride for 2 days and then concentrated *in vacuo* to dryness. Carbobenzoxy-L-aspartic anhydride crystallized on addition of ether and light petroleum and was filtered. The product was dissolved in ethyl acetate (50 ml) and added to ethyl acetate (50 ml) containing *S*-benzylcysteine ethyl ester hydrochloride (1 equiv.; 11·2 g) (Harington and Pitt-Rivers 1944) and triethylamine (2 equiv.; 11·2 ml) and allowed to stand overnight. The solution was washed successively with hydrochloric acid and water and the product finally extracted into sodium bicarbonate (0·5M) solution. On acidification this produced an oil which slowly crystallized. This was dissolved in ethyl acetate and the α- and β-isomers separated by fractional extraction into sodium bicarbonate solution (Le Quesne and Young 1952). The unwanted β-isomer had m.p. 146–147 °C and the α-isomer 114–116 °C. Yield of pure α-isomer 3 g (15% overall) (Found: N, 5·7; S, 6·5%. Calc. for $C_{24}H_{28}O_7N_2S$: N, 5·7; S, 6·6%).

(*r*) *Carbobenzoxy-*L*-asparaginyl-*S*-benzyl-*L*-cysteine Ethyl Ester.*—3 g of (*q*) in chloroform (60 ml) at —5 °C was treated with PCl_5 (1·45 g) and the solution poured into dry ether (200 ml) saturated with ammonia. The product was filtered off and extracted with hot water leaving 1·4 g (47%) of product, m.p. 174–176 °C. Recrystallization from methanol gave crystals, m.p. 188–190 °C (Found: C, 59·4; H, 6·2; N, 8·2; S, 6·3%. Calc. for $C_{24}H_{29}N_3O_6S$: C, 59·1; H, 6·0; N, 8·6; S, 6·6%). (Attempts to prepare this compound by the *iso*cyanate method of Goldschmidt and Wick (1951) were unsuccessful.)

(*s*) *S-Benzyl-*L*-cysteine Benzyl Ester Hydrochloride.*—Miller and Waelsch's (1952*b*) procedure for preparing benzyl esters gave a 66% yield of product, m.p. 148–151 °C. The salt was recrystallized from chloroform-*cyclo*hexane (Found: N, 4·1; S, 9·1%. Calc. for $C_{17}H_{20}O_2NSCl$: N, 4·2; S, 9·5%).

(*t*) *Carbobenzoxy-*L*-asparaginyl-*S*-benzyl-*L*-cysteine Benzyl Ester.*—The free *S*-benzylcysteine benzyl ester was prepared from the hydrochloride (10 g) by treatment with ammoniacal chloroform. The ammonia and chloroform were removed by vacuum distillation. The ester was not distilled, but was dissolved in dry ether (200 ml) and treated with 1 equiv. each of triethylamine and *o*-phenylene chlorophosphite. The latter was made in 79% yield by the method of Anschütz *et al.* (1943). After 1 hr the precipitated triethylamine hydrochloride was centrifuged down in stoppered tubes and washed with dry ether (100 ml). The combined ether extracts were evaporated down to give an oil. This was dissolved in 250 ml of thiophene-free benzene, anhydrous carbobenzoxy-L-asparagine (1 equiv.) added, and the mixture refluxed for 1 hr. The gel produced on cooling was filtered and dried (10 g) and then extracted with sodium bicarbonate to remove unchanged carbobenzoxyasparagine. The product was finally crystallized from ethanol, m.p. 185–187 °C. Yield 5·5 g (41% overall) (Found: N, 7·8; S, 5·8%. Calc. for $C_{29}H_{31}O_6N_3S$: N, 7·7; S, 5·8%).

(*u*) L-*Asparaginyl*-L-*cysteine*. — Prepared from carbobenzoxy-L-asparaginyl-*S*-benzyl-L-cysteinebenzyl ester (8·4 g) by reduction with sodium (2 g) in liquid ammonia followed by separation as the mercury complex with " Hopkins reagent " (Harington and Pitt-Rivers 1944) and finally as the cuprous mercaptide using cuprous oxide as in (*m*). The peptide was crystallized with difficulty from methanol-water at room temperature. (Heating in methanol-water produced a gum.) Yield 0·35 g (10% theoretical), m.p. 168·5–169 °C (Found : N, 15·9 ; S, 12·7%. Calc. for $C_{17}H_{13}O_4N_3S.H_2O$: N, 16·6 ; S, 12·7%).

(*v*) *Interaction of* S-*benzylcysteine Ethyl Ester* iso*Cyanate and Carbobenzoxyasparagine*.—*S*-benzylcysteine ethyl ester *iso*cyanate (13·4 g), purified by vacuum distillation, was refluxed with carbobenzoxy-L-asparagine (13 g) in dry dioxane (30 ml) until evolution of carbon dioxide ceased. Addition of water gave a precipitate, which was filtered off, washed with ether, and dissolved in ethyl acetate (200 ml). Extraction with sodium bicarbonate and acidification produced a white solid which had m.p. 125–127 °C after recrystallization twice from a large volume of ethanol. The compound is almost certainly bis-(α-carboethoxy-β-benzylmercaptoethyl)urea (Found : C, 59·5 ; H, 6·5 ; N, 5·6%. Calc. for $C_{25}H_{32}O_5N_2S_2$: C, 59·5 ; H, 6·4 ; N, 5·6%).

(*w*) *Carbobenzoxyasparagine Anhydride*.—Attempts to couple carbobenzoxy-L-asparagine with glycine ethyl ester by the procedure of Boissonnas (as in (*a*)) produced unchanged carbobenzoxy-L-asparagine and a substance of m.p. 124–126 °C (decomp.), which is most probably the linear anhydride (Found : C, 56·9 ; H, 5·1 ; N, 11·0%. Calc. for $C_{24}H_{26}O_9N_2$: C, 56·0 ; H, 5·1 ; N, 10·9%).

(*x*) DL-*Asparticdibenzyl Ester Hydrochloride*.—DL-Aspartic acid (0·30 mole) and toluenesulphonic acid (0·33 mole) were heated in benzyl alcohol (150 g) on an oil-bath for 1 hr at 130–140 °C. The benzyl alcohol was distilled off *in vacuo* and the resulting syrup poured into a mortar, and washed with ether (Miller and Waelsch's (1952*b*) procedure). Grinding produced a white crumbly solid (124 g), m.p. c. 106 °C. This sulphonate (98 g) was treated with chloroform-ammonia solution, the ammonium toluene sulphonate filtered off, and the free dibenzyl ester obtained as an oil on evaporation. The dry ester was added to dry ether saturated with HCl gas and the oil which separated soon solidified, giving 30 g (40% theoretical), m.p. 102–104 °C. Two recrystallizations from chloroform-*cyclo*hexane gave m.p. 108–109 °C (Found : N, 3·8%. Calc. for $C_{18}H_{20}O_4NCl$: N, 4·4%).

(*y*) *Glycyl*-DL-*aspartic Acid*.—DL-Aspartic acid dibenzyl ester toluenesulphonate (22·5 g) and chloroform-ammonia were allowed to react in methylene chloride, the ammonium *p*-toluenesulphonate filtered, and the filtrate evaporated to an oil. The ester was dissolved in methylene chloride and coupled with carbobenzoxyglycine (10 g) by the " ethyl chlorocarbonate method " as in (*a*). After effervescence had ceased, the solution was washed with HCl, water, sodium bicarbonate solution, water, and HCl. After drying, the solvent was removed by evaporation and the oil reduced by Pd-H_2 methanol in the usual way, giving 3·0 g (34% yield) peptide, m.p. 199–202 °C. Chromatography showed the presence of only one ninhydrin-reacting substance (Found : C, 37·8 ; H, 5·5 ; N, 14·6%. Calc. for $C_6H_{10}O_5N_2$: C, 37·9 ; H, 5·3 ; N, 14·7%).

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THE CHEMICAL CONSTITUENTS OF AUSTRALIAN *FLINDERSIA* SPECIES

V. THE CONSTITUENTS OF FLINDERSIA MACULOSA LINDL.

By R. F. C. Brown,* P. T. Gilham,* G. K. Hughes,* and E. Ritchie*

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Summary

From the bark of *Flindersia maculosa* Lindl. were isolated the known alkaloids, kokusaginine and flindersiamine, the new alkaloids, maculine and maculosine, the known coumarin, collinin, and a triterpene, flindissol. Maculine appears to be a methylenedioxydictamnine.

The leaves contained kokusaginine, the new alkaloid, maculosidine, and the new flavone, flindulatin. The structure 6,8-dimethoxydictamnine is proposed for maculosidine, and flindulatin is shown by degradation and synthesis to be 5-hydroxy-3,4′,7,8-tetramethoxyflavone (3,4′,7,8-tetramethylherbacetin).

I. Introduction

Of the 14 species of the genus *Flindersia* occurring in Australia (Engler and Prantl 1931) 12 are rain-forest trees and only two, *F. dissosperma* (F. Muell.) Domin. and *F. maculosa* Lindl. are found in the dry western parts of New South Wales and Queensland. The latter is a tree which may reach a height of 30–40 ft and a diameter of 12–18 in. The study of its chemical constituents was complicated by several factors. Firstly, the species showed great variability and it will be necessary in the sequel to refer to three different samples, *A*, *B*, and *C*, all collected about 30 miles west of Goondiwindi, Queensland, in April 1951, June 1952, and December 1952 respectively. Secondly, the yields of the various constituents were rather low and finally, the material itself was not readily accessible. These circumstances have rendered it impossible to clear up completely the constitutions of all the constituents but as it is unlikely that adequate supplies of material will become available for some time, it is thought worthwhile to report the results obtained so far.

From a light petroleum extract of the bark, sample *A*, the known alkaloid kokusaginine and two new alkaloids, maculosine, $C_{15}H_{15}O_5N$ (?) and maculine, $C_{13}H_9O_4N$, were isolated in a total yield of 0·036 per cent. Maculine exhibits the reactions of a furoquinoline alkaloid but maculosine appears to be of a different type. The non-basic fractions of the extract contained a new triterpene, flindissol, first isolated by Halpern (1950) from *F. dissosperma*, which is being further investigated (Professor A. J. Birch), and the coumarin derivative, collinol. Collinol (7-hydroxy-8-methoxycoumarin) is almost certainly an artefact formed by the acid dealkylation of its 7-geranyl ether, collinin, in view of the

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isolation of collinin in good yield by direct crystallization from the light petroleum extract of sample *C*.

Less than 1 kg of the bark of sample *B* was available; it was found to contain only traces of alkaloids and was not investigated further.

The bark of sample *C* yielded the alkaloids kokusaginine, flindersiamine, and maculine in a total yield of 0·003 per cent. Recrystallization of a low-melting solid which separated directly from the light petroleum extract gave collinin, first isolated from the bark of *F. collina* Bail. by Anet, Blanks, and Hughes (1949).

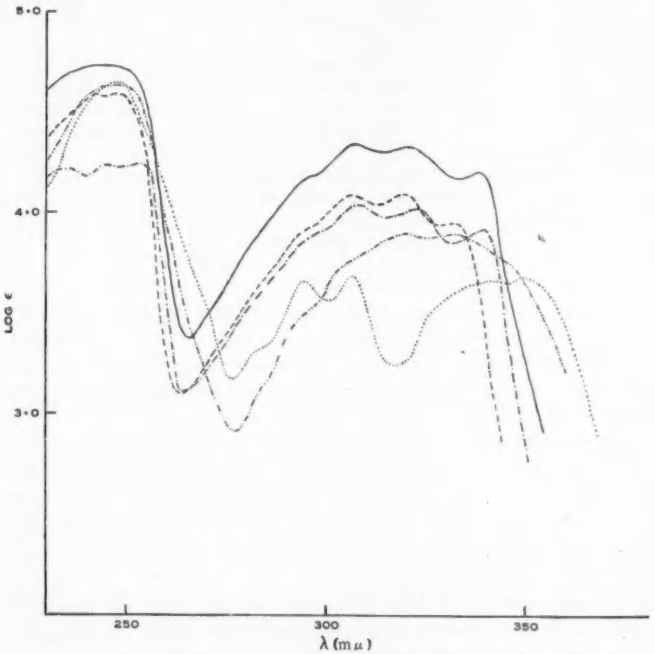


Fig. 1.—Ultraviolet spectra. —— Maculine. –·– Maculosine. ····· Maculosidine. – – – Kokusaginine. –··– Skimmianine.

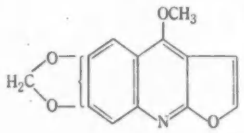
A concentrated light petroleum extract of the leaves of sample *A* on standing for several months deposited a dark waxy mass from which a new flavone, flindulatin, $C_{19}H_{18}O_7$, was isolated. The basic fraction yielded kokusaginine and a new alkaloid, maculosidine, $C_{14}H_{13}O_4N$. Vacuum distillation of the residual oil gave a small quantity of collinol.

The leaves of sample *B* contained negligible traces of alkaloids but flindulatin was obtained as before; those of sample *C* also contained only traces of alkaloids, whilst the yield of flindulatin was much smaller.

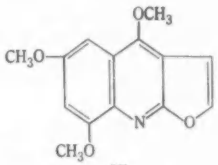
The method for the separation of the alkaloids utilized differences in the solubilities of the hydrochlorides, fractional crystallization of the free bases, and chromatography. Because of the great similarity between all the alkaloids the separations were not sharp and the difficulties were further increased by the occasional formation of constant melting point mixtures of the same type as that encountered by Cannon *et al.* (1952) in the flindersiamine-skimmianine mixture from *F. bourjotiana* F. Muell.

II. The Structures of the Alkaloids

Maculine, $C_{13}H_9O_4N$, is a weak base, contains one methoxyl group and gives a positive methylenedioxy test. Its ultraviolet absorption spectrum (Fig. 1) resembles that of kokusaginine and of skimmianine. On heating with methyl iodide under pressure it gives *iso*maculine, a weaker base, which contains no methoxyl group. This evidence suggests that maculine is a methylenedioxydictamnine (I), but lack of material has prevented further degradative work which would decide between the three alternative positions, 5,6-, 6,7-, and 7,8-, for the methylenedioxy group. Maculine is isomeric with another furoquinoline alkaloid, kokusagine, isolated by Teresaka (1933) from *Orixa japonica* Thumb. It also contains one methoxyl and one methylenedioxy group but its structure is not yet known (Ohta and Okuda 1951).



(I)



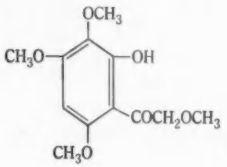
(II)

Maculosidine, $C_{14}H_{1}O_{}N$, a weak base with three methoxyl groups, has an ultraviolet absorption spectrum somewhat similar to that of kokusaginine and maculine (Fig. 1). It too is isomerized by heating with methyl iodide under pressure and the resulting *iso*maculosidine contains two methoxyl groups, so it is concluded that the alkaloid is a dimethoxydictamnine. Since *iso*maculosidine could not be demethylated by refluxing with alcoholic hydrochloric acid, although hydrolysis of the 5-methoxyl group of *iso*acronycidine was readily effected under the same conditions (Lahey, Lamberton, and Price 1950), it follows that a 5-methoxyl group is absent. Maculosidine must therefore be 6,7-, 7,8-, or 6,8-dimethoxydictamnine and, since the first two possibilities are the known alkaloids kokusaginine and skimmianine respectively, it is assigned the structure 6,8-dimethoxydictamnine (II).

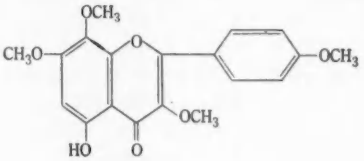
Maculosine, $C_{15}H_{15}O_5N$ (?), contained a methylenedioxy group but no methoxyl group. It gave no colour on warming with concentrated sulphuric acid. It cannot therefore be a furoquinoline alkaloid of the dictamnine type. The ultraviolet absorption spectrum is shown in Figure 1.

III. The Structure of Flindulatin

Flindulatin, $C_{19}H_{18}O_7$, contains four methoxyl groups and gives a brownish green colour with ferric chloride in alcoholic solution. Methylation gives a colourless monomethyl derivative and acetylation a monoacetate. Reduction of the pigment with magnesium and alcoholic hydrochloric acid or with sodium amalgam followed by acidification, gives a pink colour in each case, which Briggs and Locker (1951) state is characteristic of 5-hydroxy-3-methoxyflavones.



(III)



(IV)

Alkaline hydrolysis of methylflindulatin gives anisic acid and 2-hydroxy-ω,3,4,6-tetramethoxyacetophenone (III) which was first obtained by Perkin (1913) by hydrolysis of hexamethylgossypetin. On this evidence the structure 5-hydroxy-3,4′,7,8-tetramethoxyflavone (3,4′,7,8-tetramethylherbacetin) (IV) is assigned to flindulatin. In agreement with this conclusion is the fact that methylflindulatin has the same melting point as pentamethylherbacetin.

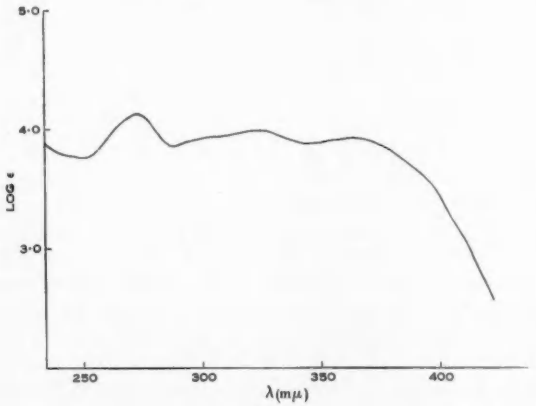


Fig. 2.—Ultraviolet spectrum of flindulatin.

Confirmation of this structure was obtained by the synthesis of flindulatin by monomethylation of 5,8-dihydroxy-3,4′,7-,trimethoxyflavone (Rao and Seshadri 1947) with diazomethane in the presence of boric acid. The natural and synthetic substances and their acetates were respectively identical. The ultraviolet absorption spectrum of flindulatin is given in Figure 2.

IV. Experimental

Melting points are uncorrected. Light petroleum refers to the fraction of b.p. 60–90 °C. Ultraviolet absorption spectra were measured in ethanol on a Beckman DU ultraviolet spectrophotometer. Analyses are by Dr. K. W. Zimmermann, C.S.I.R.O., and the late Mrs. E. Bielski, University of Sydney.

(*a*) *Extraction of the Bark.*—The dried milled bark (sample *A*, 4·9 kg) was exhausted with light petroleum at room temperature and the extract concentrated. The viscous residue was then extracted repeatedly with warm hydrochloric acid (2% ; 2 l.) and the extract filtered and basified. The liberated bases were taken up in chloroform, the solution dried, filtered through a short column of alumina to remove tars, and then evaporated to dryness. Repeated crystallization of the residue from methanol yielded colourless needles of *maculine* (1·6 g), m.p. 196–197 °C (Found : C, 64·3 ; H, 4·0 ; N, 6·0 ; OCH_3, 12·1%. Calc. for $C_{13}H_9O_4N$: C, 64·2 ; H, 3·7 ; N, 5·8 ; $1 \times OCH_3$, 12·8%).

The methanolic filtrates were evaporated to dryness and the residue dissolved in a small volume of hot 5% hydrochloric acid. The crystalline solid which formed on cooling was recrystallized three times from 2% hydrochloric acid and then converted to the free base. Recrystallization of this from ethanol yielded colourless needles of kokusaginine (0·1 g), m.p. and mixed m.p. with authentic specimen 168–169 °C (Found : C, 64·3 ; H, 5·0 ; N, 5·7%. Calc. for $C_{14}H_{13}O_4N$: C, 64·2 ; H, 5·1 ; N, 5·4%). The identification was confirmed by the preparation of the picrate, m.p. and mixed m.p. 218–219 °C.

The acid aqueous filtrates were basified and the base crystallized repeatedly from ethanol giving colourless needles of *maculosine* (0·2 g), m.p. 228–229 °C (Found : C, 61·9 ; H, 5·2 ; N, 4·7 ; OCH_3, 0·6%. Calc. for $C_{15}H_{15}O_5N$: C, 62·3 ; H, 4·8 ; N, 4·5%).

The original oily light petroleum residue from the acid extraction was washed with sodium carbonate solution, dried, and diluted with light petroleum. On standing a crystalline solid slowly separated ; this on recrystallization from benzene-light petroleum (2 : 1) furnished colourless needles of *flindissol* (5·0 g). On heating it melted at 137 °C, resolidified, and then melted again at 198 °C ; a mixture with the substance isolated from *F. dissosperma* (Halpern 1950) behaved in the same way (Found : C, 79·4 ; H, 10·0%. Calc. for $C_{30}H_{46}O_2$: C, 79·5 ; H, 9·9%).

The filtered light petroleum extract was finally freed of solvent and distilled under reduced pressure. Crude collinol distilled at 200 °C (bath temp.)/25 mm. Repeated crystallization of the solid distillate from benzene gave colourless prisms (4·4 g), m.p. and mixed m.p. with an authentic specimen 157–158 °C. The methyl ether, prepared by methylation with dimethyl sulphate and anhydrous potassium carbonate in acetone, formed colourless needles from water, m.p. and mixed m.p. with an authentic specimen 118–120 °C.

The dried milled bark from sample *C* (12·1 kg) was extracted with light petroleum as above. On concentrating the extract, however, copious separation of a greenish crystalline solid occurred. After standing for 3 days at 0° C this was collected and washed first with light petroleum and then with ice-cold ethanol. Recrystallization from ethanol gave colourless prisms of collinin (71·0 g), m.p. and mixed m.p. with an authentic specimen 66–67 °C.

The light petroleum filtrate was extracted repeatedly with warm hydrochloric acid (2% ; 3 l.) and the mixed bases isolated as above. Repeated crystallization of the mixture from methanol gave maculine (0·33 g), m.p. and mixed m.p. 196–197 °C.

The methanolic filtrates were evaporated to dryness and the residue dissolved in a small volume of hot 5% hydrochloric acid. The precipitate, which formed on cooling, was recrystallized three times from 2% hydrochloric acid. Purification of the regenerated base gave kokusaginine (0·03 g), m.p. and mixed m.p. 168–169 °C.

The acid aqueous filtrates were basified and the product crystallized repeatedly from ethanol. The crystalline solid, which had m.p. 180–185 °C and gave a greenish blue colour when warmed with concentrated sulphuric acid, was dissolved in benzene and chromatographed on a column of alumina (25 g). The column was eluted with benzene and 25 ml fractions collected. The first four fractions contained material, m.p. 180–185 °C, giving a greenish blue colour with

concentrated sulphuric acid, but material from later fractions melted above 200 °C and gave a prussian blue colour with concentrated sulphuric acid. The column was stripped by elution with chloroform and the combined later fractions evaporated to dryness. Recrystallization from ethanol gave thick colourless needles of flindersiamine (0·13 g), m.p. and mixed m.p. with an authentic specimen (Anet *et al.* 1952) 206 °C (Found : C, 61·7 ; H, 4·2 ; N, 5·4%. Calc. for $C_{14}H_{11}O_5N$: C, 61·5 ; H, 4·1 ; N, 5·2%).

(*b*) *Extraction of the Leaves.*—The dried milled leaves (sample *A*, 9·7 kg) were exhausted with light petroleum at room temperature and the extracts concentrated. After standing for 10 wk the solution had deposited a considerable quantity of dark brown solid material. The mixture was diluted with light petroleum, the waxy solid collected and washed with the same solvent. This solid (about 30 g) which contained the flavone was stirred with warm 1% hydrochloric acid to remove coprecipitated alkaloids and set aside. The light petroleum filtrate was evaporated and the residue extracted with warm hydrochloric acid (5% ; 2 l.). The combined acid extracts were filtered, basified, and shaken with chloroform. The dried chloroform solution was filtered through a short column of alumina and then evaporated. The crystalline residue was dissolved in the minimum of hot 5% hydrochloric acid and the solution allowed to cool. The precipitate was recrystallized four times from 2% hydrochloric acid, then basified, and the recovered base recrystallized from ethanol giving colourless needles of kokusaginine (0·50 g), m.p. and mixed m.p. 168–169 °C ; the picrate had m.p. and mixed m.p. 218–219 °C.

The 5% hydrochloric acid filtrate was basified, the alkaloids extracted with chloroform, and the extract after drying and filtering through a short column of alumina evaporated to dryness. Repeated recrystallization of the residue from ethanol gave small colourless plates of *maculosidine* (0·35 g), m.p. 184 °C (Found : C, 65·0 ; H, 4·9 ; N, 5·4 ; OCH_3, 32·8%. Calc. for $C_{14}H_{13}O_4N$: C, 64·9 ; H, 5·1 ; N, 5·4 ; $3\times OCH_3$, 35·9%).

The acid-washed waxy solid was dissolved in boiling ethanol, the solution charcoaled, and filtered. On cooling a difficultly filterable mixture of waxy material and flindulatin separated. Repetition of the procedure using 70% acetic acid resulted in further purification but additional recrystallizations did not improve the purity of the material. It was dissolved in chloroform (150 ml) and chromatographed on alumina (100 g). On elution with chloroform (1 l.) the waxy material passed into the filtrate leaving the pigment adsorbed as an orange band at the top of the column. The orange band was cut from the column and repeatedly extracted with boiling ethanol containing 2% of acetic acid. Evaporation of most of the alcohol and addition of water precipitated the crude pigment which was recrystallized from ethanol giving small pale yellow needles (0·50 g) of *flindulatin*, m.p. 157 °C (Found : C, 63·7 ; H, 5·1 ; O, 30·8 ; OCH_3, 33·9%. Calc. for $C_{19}H_{18}O_7$: C, 63·7 ; H, 5·0 ; O, 30·9 ; $4\times OCH_3$, 34·5%).

The thick oil (about 200 g) remaining after the extraction of the other constituents was washed with aqueous sodium carbonate, dissolved in chloroform (600 ml), and the dried solution filtered through a short column of alumina. The solvent was removed and the residual oil distilled under reduced pressure. The fraction of b.p. 140–190 °C/6 mm, which partly solidified, was drained on a porous tile and recrystallized from benzene giving colourless prisms of collinol (0·14 g), m.p. and mixed m.p. with an authentic specimen 158 °C.

Leaves from sample *B* (29·1 kg) contained only traces of alkaloids : flindulatin (2·0 g) was obtained as before, but the residual oil was not investigated.

Leaves from sample *C* (16·0 kg) also contained only traces of alkaloids and the yield of flindulatin (0·15 g) was very small.

(*c*) *Maculine.*—Maculine gave a positive Labat test for a methyldenedioxy group ; when warmed with concentrated sulphuric acid alone it gave a light blue colour. The *hydrochloride* separated as colourless prisms from 2% hydrochloric acid, m.p. 205–210 °C (decomp.) (Found : C, 56·1 ; H, 3·9%. Calc. for $C_{13}H_{10}O_4NCl$: C, 55·8 ; H, 3·6%). The *picrate* formed long yellow laths from ethanol, m.p. 198 °C (decomp.).

(*d*) iso*Maculine.*—Maculine (0·2 g) was heated with a large excess of methyl iodide for 4 hr at 100 °C. The product crystallized from benzene in colourless prisms (0·1 g), m.p. 237–239 °C (decomp.) (Found : C, 64·5 ; H, 4·1 ; OCH_3, 0%. Calc. for $C_{12}H_9O_4N$: C, 64·2 ; H, 3·7 ; OCH_3, 0%).

(*e*) *Maculosidine.*—Maculosidine gave a negative Labat test for a methylenedioxy group; it dissolved in concentrated sulphuric acid with the production of a greenish fluorescence.

(*f*) iso*Maculosidine.*—The base (0·15 g) was heated with a large excess of methyl iodide for 4 hr at 100 °C. The product crystallized from benzene in colourless needles (0·1 g), m.p. 167–168 °C (Found: C, 62·7; H, 5·3; N, 5·2; OCH_3, 22·8%. Calc. for $C_{14}H_{12}O_4N.\frac{1}{2}H_2O$: C, 62·7; H, 5·2; N, 5·2; $2\times OCH_3$, 23·1%).

When it (10 mg) was refluxed with ethanol (5 ml) and concentrated hydrochloric acid (1 ml) for 3 hr the product gave a negative ferric test. Heating its hydrochloride above its m.p. for 1 min also failed to effect demethylation.

(*g*) *Maculosine.*—Maculosine gave a positive Labat test for a methylenedioxy group, but no colour was produced on warming with concentrated sulphuric acid alone.

(*h*) *Flindulatin.*—The flavone was sparingly soluble in 10% sodium or potassium hydroxide solution. It gave a brownish green ferric test in aqueous alcoholic solution and, when reduced with either magnesium and alcoholic hydrochloric acid or with sodium amalgam followed by acidification, a pink colour was produced.

(*i*) *Methylflindulatin.*—Flindulatin, refluxed with methyl iodide and excess anhydrous potassium carbonate in acetone for 5 hr, gave the methyl ether which formed soft matted colourless needles from aqueous acetone, m.p. 158 °C (Found: C, 64·6; H, 5·5; OCH_3, 40·7%. Calc. for $C_{20}H_{20}O_7$: C, 64·5; H, 5·4; $5\times OCH_3$, 43·3%). It dissolved readily in concentrated hydrochloric acid giving a yellow solution.

(*j*) *Flindulatin Acetate.*—Acetylation with acetic anhydride and pyridine on the water-bath for 1 hr gave the *acetate* which crystallized from ethanol in very pale yellow needles, m.p. 172–173 °C (Found: C, 63·4; H, 5·4%. Calc. for $C_{21}H_{20}O_8$: C, 63·1; H, 5·0%).

(*k*) *Degradation of Methylflindulatin.*—The ether (0·15 g) was heated under reflux for 10 hr with a solution of potassium hydroxide (2 g) in water (7 ml) and ethanol (7 ml). Most of the ethanol was then boiled off, the solution cooled, acidified, and exhaustively extracted with ether. By shaking the ethereal solution with saturated aqueous sodium bicarbonate and with 5% sodium hydroxide solution, acidic and phenolic fractions respectively were obtained and were recovered by acidification and ether extraction. The acid fraction on recrystallization from water with the aid of charcoal gave colourless needles of anisic acid (0·01 g), m.p. and mixed m.p. 180 °C (Found: C, 62·9; H, 5·4%. Calc. for $C_8H_8O_3$: C, 63·1; H, 5·3%). The phenolic fraction on recrystallization from aqueous methanol (charcoal) gave pale yellow needles of 2-hydroxy-ω,3,4,6-tetramethoxyacetophenone (0·009 g), m.p. 114 °C (Found: C, 56·5; H, 6·9; OCH_3, 47·4%. Calc. for $C_{12}H_{16}O_6$: C, 56·2; H, 6·3; $4\times OCH_3$, 48·4%). Perkin (1913) gives m.p. 115–116 °C.

(*l*) *Synthesis of Flindulatin.*—(i) 3,4′-Dimethylkaempferol was prepared from ω-methoxyphloracetophenone, anisic anhydride, and anhydrous sodium anisate by the method of Rao and Seshadri (1946). It formed brown leaflets from ethanol, m.p. 229 °C (lit. 231·5 °C).

(ii) Methylation of dimethylkaempferol with dimethyl sulphate (1·1 mol.) and excess anhydrous potassium carbonate in dry acetone, or with excess of diazomethane in ether containing a little methanol and boric acid gave a product (70% yield) which formed long yellow needles from ethanol, m.p. 140–141 °C and gave a brownish green ferric test (Found: C, 66·2; H, 4·9; OCH_3, 26·5%. Calc. for $C_{18}H_{16}O_6$: C, 65·9; H, 4·9; $3\times OCH_3$, 28·3%). The melting point of this substance which could only be 3,4′,7-trimethylkaempferol was not altered even after extensive recrystallizations and chromatography, although Rao and Seshadri (1947) and Kondo and Endo (1929) report m.p. 152–153 °C. No explanation for the discrepancy can be offered.

(iii) Oxidation of trimethylkaempferol with potassium persulphate in aqueous pyridine containing potassium hydroxide (Rao and Seshadri 1947) gave 5,8-dihydroxy-3,4′,7- trimethoxyflavone which crystallized from ethanol as small yellow needles, m.p. 199 °C (lit. 196–198 °C).

(iv) Methylation of the above substance (0·08 g) with excess of ethereal diazomethane containing a little methanol and boric acid gave 5-hydroxy-3,4′,7,8-tetramethoxyflavone which

crystallized from ethanol in yellow needles (0·055 g), m.p. 167 °C alone or mixed with flindulatin (Found: C, 64·1; H, 5·2; OCH_3, 33·5%. Calc. for $C_{19}H_{18}O_7$: C, 63·7; H, 5·0; $4 \times OCH_3$, 34·5%).

The acetate had m.p. 171–172 °C alone or mixed with flindulatin acetate.

V. Acknowledgments

The authors are indebted to Messrs. J. Tracey and L. J. Webb, C.S.I.R.O., for the collection and identification of the plant samples, and to the University of Sydney for the award of a scholarship (R.F.C.B.).

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THE PARTITION SEPARATION OF TROPANE ALKALOIDS

By W. Bottomley* and P. I. Mortimer*

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Summary

Data on the partition of tropane alkaloids between an aqueous buffer phase, of varied pH, and chloroform are presented in terms of the pH at which 50 per cent. extraction occurs. The true distribution coefficient of some of the alkaloids is derived from the data.

The effect of varying the organic phase has been studied. From the data obtained, the operation of an analytical partition chromatography column is described.

I. Introduction

In the Australian plants, *Duboisia myoporoides* R.Br. and *D. leichhardtii* F. Muell., there occurs a wide range of members of the tropane group of alkaloids. The most common are hyoscine, hyoscyamine, and norhyoscyamine, but other alkaloids are occasionally present. Of these, valeroidine, tigloidine, poroidine, and *iso*poroidine have been described (Henry 1949). Hyoscine and hyoscyamine are of economic importance, but their analytical separation has presented great difficulty due to their general similarity. King and Ware (1941) described a method by which separation could be achieved, and this was developed as an analytical method by Rowson (1944). In this method the mixture of alkaloids in aqueous solution was treated with successive quantities of inorganic bases and extracted with an organic solvent. Hyoscine was extracted first and hyoscyamine was to be found in the later extracts. An adequate separation could be achieved but the possible presence of other alkaloids makes this method unsatisfactory for the determination of the alkaloids of *Duboisia* spp.

In 1948, Evans and Partridge (1948) described the separation of hyoscine and hyoscyamine by chromatography on a column of supported aqueous buffer. This method was adopted by Loftus Hills and Rodwell (1951) for use with *Duboisia* samples and later modified by Bottomley and Mortimer (1954) to enable the separation of most of the bases encountered. A series of eluants of increasing extractive power was used, and the alkaloids obtained in the order: tigloidine, hyoscine, valeroidine, hyoscyamine, norhyoscyamine. The positions of the peaks in the chromatograph were dependent on the buffer pH and on the nature and amounts of the various eluants. In the work reported in the present paper, the partition distribution of these bases between aqueous buffer and various organic solvent phases was examined. From the results obtained, it has been found possible to account for the operation of the partition column.

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II. Experimental

(*a*) *Materials*

Hyoscine hydrobromide was obtained from a commercial source.

Hyoscyamine from *D. leichhardtii* was purified by recrystallization of the sulphate from methanol-acetone (Hamerslag 1950) to constant melting point.

Valeroidine hydrobromide and tigloidine hydrobromide were a gift from Messrs. T. & H. Smith Ltd., Edinburgh, to whom our thanks are due.

Norhyoscyamine oxalate: The crude bases were extracted from selected samples of southern *D. myoporoides*; they contained almost exclusively hyoscyamine and norhyoscyamine. These alkaloids were separated by counter-current distribution and the norhyoscyamine obtained as the oxalate, which was recrystallized from *iso*propanol-water. The m.p. varied between 245 and 250 °C depending on the rate of heating (Found: C, 64·0; H, 6·7; N, 4·6%. Calc. for $C_{34}H_{44}O_{10}N_2$: C, 63·7; H, 6·9; N, 4·4%).

*apo*Atropine hydrochloride: This was prepared by the action of concentrated sulphuric acid on hyoscyamine sulphate, as described by Hesse (1893). The crude hydrochloride was recrystallized from water (2 vol.) avoiding excessive heating, to constant m.p.; m.p. 247 °C (uncorr.). Picrate, m.p. 168 °C.

Belladonnine sulphate was obtained for us by Mr. H. A. Berens of Biddle, Sawyer & Co. Ltd., London, whom we wish to thank for this kindness.

(*b*) *Methods*

All measurements were made at room temperature, 19–23 °C.

Distribution behaviour was studied using potassium phosphate buffers, sp. gr. 1·15, and chloroform B.P. The buffer pH was measured with a glass electrode pH meter, and the phases were mutually presaturated. Equal volumes were used, and the concentration of alkaloid (introduced as free base) was about 0·5 per cent. with respect to the total volume. With *apo*atropine, a solid phase was present at this concentration, and for this base, therefore, the concentration was halved. After equilibration the alkaloid in an aliquot of the organic layer was titrated with 0·01N *p*-toluenesulphonic acid in chloroform, using dimethyl yellow as indicator. Successive determinations showed good agreement, and the results presented in Table 1 are probably within 0·05 pH units of the correct values. Nicotine and nornicotine did not titrate accurately, the equivalence varying with the volume, and the end-point was very poor. However, as the distribution was carried out at the pH at which 50% extraction occurs, relatively large errors in the titration did not greatly affect the result.

The same procedure was used with the other solvents tested. Hyoscyamine and hyoscine only were used in this part of the work. Where the solvent interfered with the titration, for example, ether, it was evaporated and the titration carried out in chloroform.

Some pK_a values were available from the literature. Values obtained in the present work were calculated from pH readings obtained by means of a glass electrode pH meter, during titration of 2% alkaloid salt solutions with sodium hydroxide, in the interval 0·3–0·7 neutralization. The pK_a results

are thought to be accurate within 0·03 pH units. The insolubility of tigloidine and *apo*atropine in water necessitated the use of 50% ethanol as solvent. As indicated by Saunders and Srivasta (1951) the pK_a values are then about 0·4 units lower than in water.

Paper chromatography was carried out on Whatman No. 1 paper buffered with Sorensen buffers, using butanol equilibrated with buffer as moving phase and 100μg alkaloid per spot, as described by Brindle, Carless, and Woodhead (1951).

III. Results and Discussion

(a) *Partition Distribution between Chloroform and Aqueous Buffer Phase*

Orchin and Golumbic (1950) have discussed the relationship between the pH of the aqueous phase and the observed distribution coefficient k' for a monacid base in a simple distribution system, where the base occurs in the

Table 1

DISTRIBUTION BEHAVIOUR OF DUBOISIA ALKALOIDS

Alkaloid	$pH_{\frac{1}{2}}$ Chloroform/ Buffer	pK_a in Water	pK_a in 50% Ethanol	Log k
Hyoscyamine	7·00	9·68 9·65*	9·22 9·10†	2·68
Hyoscine	5·48	7·62 7·55* 7·57†	7·23 7·15†	2·14
Norhyoscyamine	7·89	10·28	—	2·39
Tigloidine	5·08	(9·3)‡	8·88	4·2
Valeroidine	6·67	—	—	—
*apo*Atropine	4·65	(9·4)‡	8·98	4·7
Nicotine	5·7–6·0	3·04, 7·84*	—	—
Nornicotine	7·63	—	—	—

* Schoorl (1939).

† Calculated from Saunders and Srivasta (1951).

‡ Figures inferred from pK_a value found using 50 per cent. ethanol.

organic phase as the non-ionized and unassociated base only. They have shown that if k is the true distribution coefficient of the free base,

$$\log k' = \log k + pH - pK_a, \quad \ldots\ldots\ldots\ldots \quad (1)$$

for pH values much lower than the pK_a of the base. The tropane alkaloids have been found to follow this relationship in the systems studied, and their partition behaviour may be characterized by the pH at which $\log k' = 0$ (i.e. when 50 per cent. extraction occurs with equal volumes of phases). For this figure, the symbol $pH_{\frac{1}{2}}$ is used, following its use by Irving *et al.* (1949) in a similar connection.

The true distribution coefficient k may be calculated for those alkaloids whose pK_a values are available. If equation (1) holds at the pH at which $\log k'=0$ (i.e. $pH=pH_{\frac{1}{2}}$), then, substituting these values in that equation, and rearranging, we have

$$\log k = pK_a - pH_{\frac{1}{2}}. \quad \ldots\ldots\ldots\ldots\ldots\ (2)$$

In Table 1 are presented the $pH_{\frac{1}{2}}$ values obtained using buffer-saturated chloroform as the organic phase, together with the pK_a values and the derived figures for $\log k$. Data for the alkaloids nicotine and nornicotine which also occur in the Solanaceae, are included for comparison. The pK_a values for nornicotine are not available, but it behaves as a monacid base at about its $pH_{\frac{1}{2}}$ value. Discordant results were obtained for the $pH_{\frac{1}{2}}$ of nicotine.

Many apparent distribution coefficients were determined by Collander (1949) who attempted a comparison on the basis of chemical constitution, with some success. But for such comparison it is preferable to use the true distribution coefficient, k, which isolates the effect of a substituent group on the distribution coefficient from any effect it may have on the basicity of the compound. For example, the presence of the epoxide ring in hyoscine reduces the true distribution coefficient by a factor of 3·5, but when apparent distribution coefficients are considered, this is concealed by its effect on the ionization of the base. An attempt to correlate chemical constitution and distribution behaviour has been made by Bate-Smith and Westall (1950) and their "R_M" value has the same dimensions as $\log k$.

Another point arises from consideration of the data presented in Table 1. In examination of crude alkaloid mixtures, it has been customary, for convenience, to refer to the bases first extracted from mixtures in aqueous solutions as "weak" bases (e.g. Schlittler, Heusler, and Friedrik 1949). This usage is inaccurate, for example, tigloidine is a considerably stronger base than hyoscine, but is more readily extracted.

The distribution behaviour of the pyridine alkaloids in a similar phase system has been examined by Badgett, Eisner, and Walens (1952).

A knowledge of the $pH_{\frac{1}{2}}$ values is useful when a mixture of bases is to be separated by Craig distribution. Where the phase volumes are equal, most efficient separation of any two bases is obtained at the pH where the geometric mean of the apparent distribution coefficients is unity (Craig and Craig 1950). If the concentration of alkaloid is low enough not to raise the buffer pH appreciably, this condition occurs when the buffer pH is the average of the $pH_{\frac{1}{2}}$ values of the two bases.

The $pH_{\frac{1}{2}}$ values of hyoscine and hyoscyamine are sufficiently far apart to allow adequate separation to be obtained by the technique of King and Ware (1941).

(b) Effect of Change of Solvent on Apparent Distribution Coefficient

The change in the real distribution coefficient, when changing to a solvent other than chloroform, may be measured by the change in the apparent distribution coefficient and expressed as the change in $pH_{\frac{1}{2}}$ value from that obtained

with chloroform as the organic phase. This has been taken as a measure of the relative strengths of the solvents as extractants, and experimentally determined data are shown in Table 2. In the following, it has been assumed that a particular

TABLE 2

CHANGE IN $pH_{\frac{1}{2}}$ VALUES WITH ORGANIC SOLVENT

Solvent	Hyoscyamine	Hyoscine
Chloroform	Standard	Standard
Chloroform : ether 40 : 60	1·0	—
Chloroform : ether 20 : 80	1·2	—
Ether	1·5	1·5
Ether : light petroleum 50 : 50	2·1	2·3
Light petroleum (b.p. 40–60 °C)	—	3·0
Trichloroethylene	1·1	—
Dichloromethane	0·5	—
1,2-Dichloroethane	0·9	—
Ethyl acetate	1·0	—
Butanol : chloroform 20 : 80	—0·7	—

solvent change will bring about the same change in $pH_{\frac{1}{2}}$ value for each member of the series of the closely related tropane alkaloids.

(*c*) *Use of Multiple Eluants in Partition Columns*

From a partition column containing aqueous buffer as the stationary phase, the tropane alkaloids are eluted in the order of their $pH_{\frac{1}{2}}$ values. The position of the peak for each alkaloid in the chromatograph is a complex function of the $pH_{\frac{1}{2}}$ value, dependent on the eluant series used and the pH of the buffer used. Figure 1 shows the curve for this function, calculated for a column and conditions similar to those used in the assay of *Duboisia* samples. The basis for the calculation is given in the following discussion :

It is assumed that the partition column operates as described by Bock (1950), whose use of the term p to denote the portion of solute in any region present in the mobile phase (as also used by Stene (1944)) is followed here. To simplify treatment, the increase in buffer pH by the alkaloid is neglected.

The partition behaviour of the monacid bases has been characterized above by the $pH_{\frac{1}{2}}$ value in an arbitrary standard system. From this figure, there may be obtained for such a base, the value of p for this base when the phases are changed to those present in the column. If the peak concentration of the base is eluted in the first eluant, the fraction in which it occurs is related to the appropriate value of p by the expression :

$$\text{fraction number} = \left(\frac{1}{p} \times \text{number of fractions of eluant in column} - 1\right).$$

However, if the peak concentration is not eluted by the limited amount of first eluant, the peak will be carried down the column the distance

$$Nm = N.p,$$

where Nm is the distance measured in arbitrary lengths and N is the amount of eluant applied, expressed in terms of the volume of eluant in such lengths of column.

Application of the second eluant displaces the Nm portions of first eluant remaining above the peak, which then carry the peak further down the column. It may be shown that the distance the peak is carried down the column by the first eluant, and before it is reached by the second eluant, is given by

$$M = N.k',$$

where M is the distance measured in arbitrary lengths as above, and k' is the apparent distribution coefficient of the base.

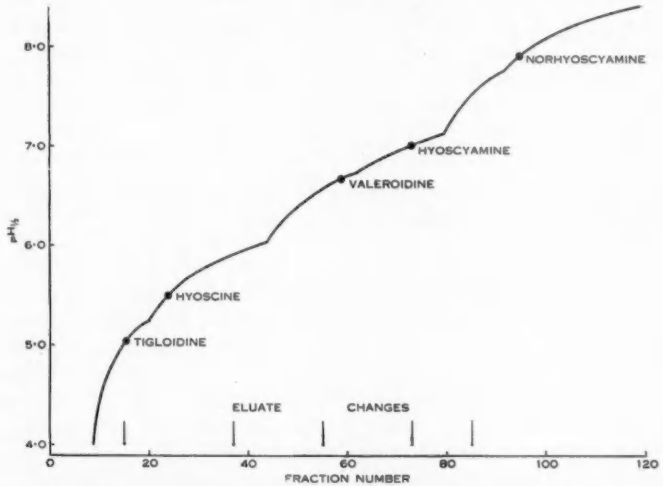

Fig. 1.—Relation between $pH_{\frac{1}{2}}$ and position of peak in a normal chromatograph of tropane bases. Curve calculated as described in text. Normal positions of peaks for common *Duboisia* alkaloids are indicated.

By following the effect of the various eluants in this manner, the position of a base in the chromatogram may be derived from its $pH_{\frac{1}{2}}$ value. The curve shown in Figure 1 has been calculated for the eluant series used in the analytical separation (Bottomley and Mortimer 1954), for a column holding seven fractions of eluate.

On account of variations in the volume of moving phase in the column, and other operating variables, there is a small variation in the positions of the peaks in chromatographs obtained in the assay work. The normal positions of the peaks when the alkaloids were present in small quantities are indicated in Figure 1. (For this purpose, the $pH_{\frac{1}{2}}$ scale, which was not fixed in the above derivation of the curve, has been located to correspond to the $pH_{\frac{1}{2}}$ value obtained in the standardized system above.) In practice, hyoscyamine was present in

such quantity in many samples that the peak was advanced because the pH of the buffer was raised by the concentration of hyoscyamine.

The calculation does not hold rigidly for hyoscine, for which equation (1) is not valid at the pH used (pH 7·8). Although nornicotine was obtained in the position corresponding to its $pH_{\frac{1}{2}}$ value, nicotine was always obtained in the first portions of eluant passed through the column.

If a single eluant is used in a partition chromatogram, satisfactory separation is obtained over a small $pH_{\frac{1}{2}}$ range (about 1·0 pH units), and the peak height falls off rapidly. When an unknown mixture is to be examined, it is desirable that separation be as uniform as possible over an extensive $pH_{\frac{1}{2}}$ range. It is seen that this state was approached with only five eluant changes. Stein and Moore (1951) have similarly been able to maintain peak definition in extended chromatographs, by the use of multiple eluates.

(*d*) *Chromatography on Buffered Paper*

It was hoped that chromatography of the alkaloids on buffered paper would provide information on the relative $pH_{\frac{1}{2}}$ values of the alkaloids, but it is apparent from the results (Table 3) that the order of movement of these bases on buffered

TABLE 3

R_F VALUES ON BUFFERED PAPER

pH	6·2	6·8	7·4	8·0
*apo*Atropine	0·48	0·47	0·53	0·58
Belladonnine	0·11	0·11	0·16	0·15
Hyoscine	0·42	0·53	0·62	0·67
Hyoscyamine	0·34	0·35	0·38	0·39
Norhyoscyamine	0·37	0·36	0·35	0·41

paper differs from the order of their $pH_{\frac{1}{2}}$ values (Table 1). This may be due to the introduction of other effects by the much more polar solvent used for the paper chromatography, or it may be evidence that paper chromatography is not true partition chromatography.

Chromatography on buffered paper had been carried out by Brindle, Carless, and Woodhead (1951) who reported the movement of *apo*atropine to be much less than hyoscine, or even hyoscyamine, contrary to its behaviour on our partition columns.

It was found that, when the free base was prepared from *apo*atropine hydrochloride without exposure to heat, it gave a spot at about the same position as hyoscine, but exposure of the free base to heat resulted in conversion to material giving an elongated spot in the position ascribed to *apo*atropine by Brindle, Carless, and Woodhead. Belladonnine (Küssner 1938) was found to give a normal-shaped spot with the same R_F as the faster moving portion of the material obtained by heating *apo*atropine ; this R_F value is given in Table 3.

The transformation of *apo*atropine takes place very readily when the free base is exposed to heat, and it appears that it had occurred with the *apo*atropine used by Brindle, Carless, and Woodhead.

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SHORT COMMUNICATIONS

IMPROVED TECHNIQUE FOR OSCILLOGRAPHIC STUDIES OF ELECTRODE PROCESSES: THE REDUCTION OF CHROMIUM COMPOUNDS AND OF HYDROGEN PEROXIDE*

By J. H. GREEN†

A method of observing and recording alternating potential-time $E-t$ and first time derivative of the potential-time ($dE/dt-t$) relations at an electrode passing a constant current was begun by Heyrovský and Forejt (1943). This technique and succeeding applications of it suffered from several drawbacks; the $E-t$ and $dE/dt-t$ curves were recorded separately and then aligned for comparison; the curves displayed on the oscilloscope screen varied with the growth of the mercury drop electrode making it impossible to record the "derivative" curve properly or requiring the use of the rather cumbersome streaming electrode. Up to the present, all experiments have been carried out with a mercury electrode.

An attempt has been made to improve the technique in two ways which are reported here. In the first place, both the $E-t$ and the $dE/dt-t$ curves are shown and photographed simultaneously on the screen of a double-beam oscilloscope. In the second place, solid metallic microelectrodes have been used to extend the basic information available for technical electrodeposition and to obtain steady "derivative" patterns on the oscilloscope screen.

Apparatus and Technique

A conventional H-cell was used with a saturated calomel half-cell as the reference anode. In general, de-aeration of solutions is unnecessary since oxygen does not affect the interpretation of the oscilloscope patterns. A dropping mercury electrode with a slow drop-rate is required since the A.C. impulses cause rapid oscillations of the drop and may cause the mercury to emerge as a stream of fine drops. Platinum microelectrodes are sealed into glass tubes, cleaned in nitric acid, and anodically cleaned in dilute sulphuric acid before use.

The electrical circuit used to supply constant current impulses from the A.C. supply, together with a means of altering the D.C. potential at the cathode and of recording $E-t$ and $dE/dt-t$ curves on the oscilloscope, is shown in Figure 1. The modifications which were required for removal of the switch

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circuit and for the appropriate connections to the double-beam oscilloscope may be seen by comparing the circuit given by Heyrovský and Forejt (1943). The oscilloscope is an A. C. Cossor, London, model 1049 type, and patterns on the screen are recorded by photographing them on 35 mm Super XX film in a camera designed for the instrument.

To measure the potentials at which the various electrode reactions occur, one may use one of three procedures : firstly, D.C. potential may be adjusted so that the A.C. potential just reaches the potential of a time-lag on the $E-t$ curve, and the electrode potential may then be measured with a potentiometer. Secondly, an inherent advantage of the present modified technique is that either of the two curves may be moved vertically on the screen until the points of inflexion on the derivative curve coincide with the $E-t$ curve. If a photograph

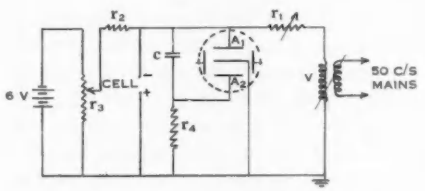

Fig. 1.—Circuit for electrolyses with A.C. superimposed on D.C., and for the display of E–t and dE/dt–t (after RC differentiation) curves on an oscilloscope screen. $r_1=1$ MΩ ; $r_2=10{,}000\Omega$; $r_3=30\Omega$; $r_4=1000\Omega$; $c=0{\cdot}03$ μF ; $V=100$–200 V (r.m.s.).

is taken of this pattern it is possible to fix the positions of indistinct time-lags quite definitely. This is shown in Figure 2*G*. Thirdly, the separated curves may be recorded and the appropriate lines may be drawn from the derivative to the $E-t$ curve. Photographs may then be enlarged and superposed on a graduated scale (e.g. graph paper) for measurement.

The limits of the potential-time curves are those of the saturated calomel electrode and of the limiting cathodic process. For example,

$$K^+ + e \rightarrow K,$$

at the mercury electrode in KCl or KOH, or

$$H^+ + e \rightarrow \tfrac{1}{2}H_2,$$

at the platinum electrode.

Figure 2 reproduces tracings of the curves which are shown about $1\frac{1}{2}$ times their size on the oscilloscope screen. The potentials calculated are based on ranges for the potential ordinates of 0 to $-1{\cdot}9$ V (*versus* the saturated calomel electrode) for the dropping mercury electrode and 0 to $-1{\cdot}26$ V for the bright platinum electrode. In general, the potentials stated for the various processes will be as much as 10 per cent. in error, since a precise knowledge of the overpotentials of the cathodic processes which determine the full range is lacking.

A further comment on the nature of the curves is necessary since the exact fixing of the potentials of electrode processes from the $E-t$ curves depends on it. The $E-t$ curve can be visualized as representing the charging and discharging of a leaky condenser (the electrical double layer). Thus, when a negative potential is applied across the electrolytic cell, the negative potential of the cathode increases at a rate determined by the sinusoidal variation of the alternating current. However, when a potential is reached at which an electroreduction will occur, electrons are used up in the reduction, and for a time the electrode potential remains stationary. This appears as a " time-lag " on the $E-t$ curves, and it is repeated as a decrease in charging (capacity) current on the derivative curves. When the reducible ions in the double layer are exhausted the potential of the electrode increases again to the potential limit. The sign of the current impulses then reverses and the pattern is repeated in the opposite sense. The slopes of the two sides of the $E-t$ curves will be the greater the greater the current impulse through the cell (i.e. the smaller the value of r_1 in Fig. 1 at constant V).

The ordinate of the derivative curve is a measure of the capacity current (i_C) at the cathode. The total current, which is constant, is given by

$$i=i_C+i_E,$$

where i_E is the current due to any electron transfer at the electrode. An increase in i_E will cause a decrease in i_C shown as a dip towards the zero axis of the derivative curve. Thus at point b in Figure 2B a reduction has just begun and at point d it has reached its maximum rate and gradually decreases to e. Where an electrode reaction is relatively slow the points b and d will occur at measureably different potentials, but in general it is probably best to refer to a single potential within the limits of error of the measurement. This is taken to be the potential of point d at which the electrode process is occurring at its fastest. For comparative purposes it can be expected that the potential of point d (E_m) will be somewhat more negative than the half-wave potential ($E_{\frac{1}{2}}$) in the D.C. polarogram of the same system.

Results

Some preliminary results are given here to illustrate the method. The indifferent electrolytes which are employed in the electrolyses are more concentrated, usually molal, than in orthodox polarography to decrease the iR drop in the solution. An experiment is normally carried out by observing the $E-t$ and $dE/dt-t$ curves for 10 c.c. of the indifferent electrolyte and then for the solution obtained by adding two drops of a 10^{-1}M solution of the material to be investigated. Reagent grade materials are used throughout the experiments.

Chromate ion shows a strong cathodic step in alkaline solution and an anodic process both with $E_m=-1{\cdot}04$ V ($E_{\frac{1}{2}}=-0{\cdot}85$ V; Lingane and Kolthoff 1940). Chrome alum, presumably in the form of CrO_2^- ion, is reduced under the same conditions at $E_m=-1{\cdot}1$ V, a reduction which has not been observed in D.C. electrolyses. It is interesting to note that, at a bright platinum electrode, one cathodic step is observed for chromate ion at $E_m=-0{\cdot}78$ V and there is no

anodic step. The beginning of the reduction is at $-0\cdot63$ V which is close to the equilibrium $CrO_4^{=}/CrO_2^{-}$ potential at pH=14 ($-0\cdot5$ V), and the E_m value is $0\cdot26$ V closer to the equilibrium potential at a platinum than at a mercury

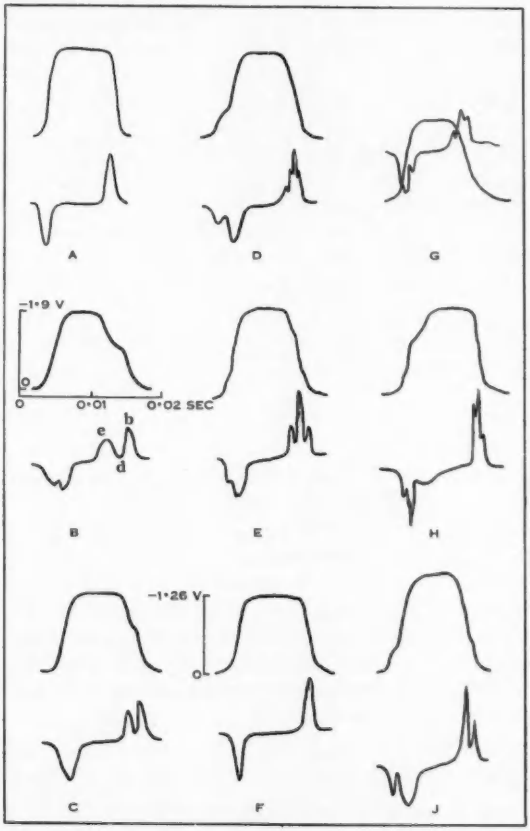

Fig. 2.—*E–t* and d*E*/d*t*–*t* curves traced from enlargements of 35 mm photographs. *A*, 1M KOH, D.M.E. ; *B*, 1M KOH, K_2CrO_4, D.M.E. ; *C*, 1M KOH, chrome alum, D.M.E. ; *D*, 1M KCl, chrome alum, D.M.E. ; *E*, 1M KOH, H_2O_2, D.M.E. ; *F*, 1M KOH, bright platinum ; *G*, 1M KOH, H_2O_2, bright platinum ; *H*, 1M KCl, K_2CrO_4, D.M.E. ; *J*, 1M KCl, K_2CrO_4, $MgCl_2$, D.M.E. Right-hand side of *E–t* curve is cathodic (negative potential increasing upwards) and the left-hand side is anodic.

electrode. If chrome alum solution is added to slightly acid potassium chloride an interesting curve appears. Three cathodic processes are recorded at $E_m=-0\cdot61$, $-1\cdot27$, and $-1\cdot6$ V and a single anodic step at $-0\cdot55$ V. The first two potentials may be compared with those for the ions $Cr(OH)_2^{+}$ at

$E_{\frac{1}{2}} = -0{\cdot}57$ V, and $Cr(H_2O)_6^{3+}$ at $E_{\frac{1}{2}} = -1{\cdot}05$ V (cf. Reynolds, Shalgosky, and Webber 1953) and the third potential is about 0·3 V more negative than the calculated value for the reduction of divalent chromium (unpublished recalculation of the thermodynamics of the Cr-H_2O system following Pourbaix (1948)). A third wave at $E_{\frac{1}{2}} = -0{\cdot}87$ V is found in fresh solutions and is ascribed to the reduction of the $Cr(OH)^{++}$ ion.

In the reduction of a solution of potassium chromate in potassium chloride at pH=6·8 two cathodic and two anodic steps are observed at $E_m = -0{\cdot}48$ and $-1{\cdot}4$ V (cathodic) and $-1{\cdot}2$ and $-0{\cdot}44$ V (anodic). These potentials correspond to the half-wave potentials $-0{\cdot}3$ and $-1{\cdot}3$ V for the reduction of $HCrO_4^-$ and $CrO_4^=$ ions respectively (Green and Walkley, unpublished data 1954). Both reactions seem to be slow, but the most positive reduction (of $HCrO_4^-$ ions) is less irreversible. If magnesium chloride is then added to the same solution, a striking change is found. One step only is obtained on the $E-t$ curve at $-0{\cdot}6$ V, and it is reversible. This is shown in Figures 2*H* and 2*J*. The experiment was designed to investigate the effect of Mg^{++} ion on the D.C. polarogram of a neutral chromate solution. Lingane and Kolthoff (1940) noted that the polarogram showed a wave at $-0{\cdot}2$ V followed by a more or less gradual increase of current. The author has found that Mg^{++} ion has no effect until it reaches a certain minimum concentration which appears to be less when the A.C. technique is used than it is when using D.C. It has been noticed also that the effect can be nullified by raising the temperature of the electrolyte, for example, from 15 to 23 °C. The same effect does not occur when Ca^{++} and Sr^{++} ions are substituted for Mg^{++}, but the presence of $H_2PO_4^-$ ions does give an effect resembling that of Mg^{++}. It is not yet possible to do more than speculate about the reason for the Mg^{++} ion effect. It may perhaps be the result of an ion-association of the form $MgHCrO_4^+$, which would involve all hexavalent chromium ions by displacement of the chromate-acid chromate equilibrium. This would account for the disappearance of the second reduction process, and the reduction of the unipositive ion would occur more readily (i.e. more reversibly) than the reduction of $HCrO_4^-$ or $CrO_4^=$ ions.

The remaining curves (Figs. 2*E*, 2*F*, and 2*G*) are typical of those obtained with hydrogen peroxide (or the HO_2^- ion) in alkaline solution at mercury and platinum electrodes. At both electrodes two cathodic and two anodic steps are observed, which cannot be ascribed either to the presence of oxygen, since aerated and de-aerated solutions behave in the same way, or to the presence of reducible or adsorbable impurities. In D.C. polarography of the same solutions it had been observed (Kolthoff and Lingane 1952) that a single, highly irreversible step was obtained which corresponded to the 2-electron reduction

$$H_2O_2 + 2e \rightarrow 2OH^-.$$

Indications had been found, however, of a double wave in the presence of divalent magnesium and manganese ions (Juliard 1950 ; Rysselberghe and Murdoch 1950). The splitting of the peroxide wave may well have been due to a catalytic effect of Mg^{++} and Mn^{++} ions similar to that found in other systems (Kolthoff and Lingane 1952, p. 281).

With the present technique the E_m values of the steps observed are $-0{\cdot}41$ and $-1{\cdot}37$ V (cathodic) and $-1{\cdot}37$ and $-0{\cdot}6$ V (anodic) at the mercury electrode. At the platinum electrode the potentials are $-0{\cdot}82$ and $-1{\cdot}18$ V (cathodic) and $-0{\cdot}99$ and $-0{\cdot}21$ V (anodic). The most negative step at both electrodes is " oscillographically reversible ", that is, occurring at the same potential. Further, the areas of the " cut-ins " on the derivative curve for the mercury electrode, which are the most definite, are equal on the cathodic branch, suggesting that the same number of electrons is required for each reduction process. The results can then be explained by considering that two successive electrode processes occur as the potential becomes more negative :

$$H_2O_2 + e \rightarrow OH + OH^-,$$

and

$$OH + e \rightarrow OH^-.$$

Such a mechanism as this has been suggested many times in the last 20 years (cf. Uri 1952), but the present results appear to give the first direct electrochemical evidence of its validity.

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A NOTE ON THE HELFERICH REACTION*

By M. A. JERMYN†

Helferich and Schmitz-Hillebrecht (1933) showed that phenols will condense with fully acetylated aldose sugars in the presence of suitable acid catalysts to give the acetyl derivatives of arylglycosides. The reaction product normally contains considerable quantities of both the α- and β-isomers (Williams 1940) and a great deal of manipulation has usually been involved in obtaining crystalline products, whose constitution cannot be known *a priori*, although certain conditions favouring the formation of α- or β-glycosides have been worked out.

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In the course of an investigation directed towards simplifying the synthesis of the *p*-nitrophenylglucosides which are commonly used as substrates for the detection and estimation of glucosidases it has been found that certain characteristic solubility differences exist for the α- and β-arylglucosides and their tetra-acetates which can be used to make the separation much easier. The tetra-acetyl aryl-β-glucosides have been found to be in general much less soluble in ethanol than their α-isomers, while the aryl-α-glucosides are much less soluble than the β-isomers. These differences are enhanced in ethanolic solutions of crude reaction mixtures and only the tetra-acetyl β-glucoside can be crystallized directly from a solution of the reaction melt in two volumes of ethanol. This crystallization proceeds quite readily once seed crystals are available.

TABLE 1

YIELDS OF VARIOUS TETRA-ACETYL ARYL-β-GLUCOSIDES FROM THE HELFERICH REACTION BY DIRECT CRYSTALLIZATION OF THE REACTION MELT FROM ETHANOL

Catalyst, *p*-toluenesulphonic acid

Phenols	Reaction Temperature (°C)	Yield (%)
Phenol	100	33
o-Cresol	100	37
Thymol	100	18
6-Bromo-2-naphthol	100	21
m-Cresol	100	33
2-Naphthol	130	39
p-Chlorophenol	100	44
o-Nitrophenol	130	61

For *m*- and *p*-nitrophenol it has been found that the usual catalysts for producing an excess of the β-isomer, such as *p*-toluenesulphonic acid, or sulphuric acid, are quite ineffective catalysts for the condensation, penta-acetyl β-glucose being recovered unchanged from the reaction mixture. Zinc chloride, a catalyst which usually leads to a large excess of the α-isomer, is effective at slightly elevated temperatures and with *p*-nitrophenol it is possible to obtain reasonable yields of both tetra-acetyl *p*-nitrophenyl-β-glucoside and *p*-nitrophenyl-α-glucoside. The separation is aided by the almost quantitative insolubility of the tetra-acetyl β-glucoside in ethanol. This property has enabled an investigation to be made of the effect of various factors on the yield of the reaction.

Zinc chloride, which is insoluble in the reaction mixture, appears to act as a heterogeneous catalyst for a spontaneous initial reaction. The relative amounts of the α- and β-isomers formed depend on the reaction temperature, the ratio of the reactants, and the amounts of catalyst. This initial equilibrium is slowly displaced away from the β-isomer on further heating. The low yields of *p*-nitrophenyl-β-glucoside obtained from the zinc chloride-catalysed reaction by Helferich and Peters (1933) and Aizawa (1939) are explainable by the low reaction temperatures (115–120 and 100 °C respectively) which they used.

Experimental

(*a*) *Preparation of Tetra-acetyl Aryl-β-glucosides.*—Equal weights of penta-acetyl β-glucose and the phenol are condensed together by heating for 30 min in the presence of a catalytic amount of *p*-toluenesulphonic acid (0·2 g/100 g) with the continuous removal of acetic acid by vacuum distillation (Sisido 1936). Two volumes of ethanol are added to the hot reaction mixture as quickly as possible and the ethanolic solution is then put aside in the refrigerator to crystallize.

A selection of compounds prepared, reaction temperatures, and yields are shown in Table 1.

(*b*) *Preparation of Tetra-acetyl* p-*Nitrophenyl-β-glucoside and of* p-*Nitrophenyl-α-glucoside.*—The reaction conditions given below are not optimum for either product. A mixture of 100 g of *p*-nitrophenol and 100 g of penta-acetyl β-glucose were fused together in a litre flask, allowed to come to bath temperature (140 °C), 4 g of powdered $ZnCl_2$ added all at once, and the pressure reduced. A spontaneous reaction set in after 1–2 min. After a total of 15 min heating, the flask was disconnected from the pump, 400 ml of ethanol added, and the crystallization allowed to proceed for 18 hr. The crystalline tetra-acetyl β-glucoside was filtered off and washed well with cold ethanol. Yield 38·9 g (31%), contaminated with a little tar. Recrystallization from a solution in ethanol-acetone (3 : 2) decolorized with charcoal gave white needles, m.p. 176° C (uncorr.). The usual value given for the m.p. is 174–175 °C (cf. Glaser and Wulwek 1924 ; Latham, May, and Missetig 1950).

TABLE 2

EFFECT OF VARIOUS FACTORS ON THE YIELD OF TETRA-ACETYL *p*-NITROPHENYL-β-GLUCOSIDE FROM THE HELFERICH REACTION

100% Yield = 30 g throughout

(*a*) Temperature

25 g β-PAG, 25 g *p*-nitrophenol, 1 g zinc chloride ; reaction time 30 min

T (°C)	Yield (%)	Remarks
120	—	–PAG recovered unchanged
130	—	Semi-crystalline mixtures containing a little β-glucoside
140	36·3	
160	42·3	This is the temperature of maximum yield for reaction times from 7·5 to 60 min
180	25·0	Excessive tar formation

(*b*) Reaction Time

25 g β-PAG, 25 g *p*-nitrophenol, 1 g zinc chloride ; reaction temperature 160 °C

Reaction Time (min)	Yield (%)
7·5	47·0
15	45·8
30	44·3
60	37·0

TABLE 2 (*Continued*)

(*c*) Ratio of Reactants
25 g β-PAG, 1 g zinc chloride ; temperature 160 °C ; reaction time 15 min

-PAG/*p*-Nitrophenol (mol ratio)	Yield (%)
1·4	37·7
2·1	42·0
2·8*	45·8
4·2	38·7
5·6	—

(*d*) Amount of Catalyst
25 g β-PAG, 25 g *p*-nitrophenol ; temperature 160 °C ; reaction time 7·5 min

Weight of Zinc Chloride (g)	Yield (%)
0·2	37·0
0·5	42·1
1·0	47·0
2·5	29·3
5·0	22·0

* Equal weights.

Benzene (200 ml) was added to the combined filtrate and washings, which had been centrifuged to remove a little tar, and the benzene layer formed after the addition of water in excess was washed repeatedly with water, dilute alkali, and again with water. Excess of ethanol was added and the mixture distilled through a short column until the temperature indicated that substantially pure ethanol had been passing for some time. The resulting anhydrous ethanol solution was cooled and the solute catalytically deacetylated by the addition of a little sodium. *p*-Nitrophenyl-α-glucoside began to separate almost immediately and after standing overnight in the refrigerator the crude product was filtered off and well washed with cold ethanol. Yield 28 g (36%). Recrystallization from methanol after decolorization with charcoal gave white needles, m.p. 214 °C (uncorr.). (Montgomery, Richtmeyer, and Hudson (1942) gave 216 °C.) Using a reaction procedure identical with the above at 160 °C tetra-acetyl *m*-nitrophenyl-β-glucoside (which has not been previously synthesized by the Helferich method) was isolated in 15% yield, m.p. 136 °C, and tetra-acetyl *p*-nitrophenyl-β-galactoside, m.p. 144 °C, in 54% yield.

(*c*) *Factors Governing the Yield of Tetra-acetyl* p-*Nitrophenyl-β-glucoside.*—A standard procedure was adopted in which 25 g of penta-acetyl β-glucose was reacted in a 250 ml flask. A maximum theoretical yield of 30 g of the nitrophenyl compound could be expected in all cases. The results are set out in Table 2. The scale on which the reaction is carried out has a considerable influence on yield ; if the optimum conditions (160 °C, 7·5 min heating, PAG/nitrophenol ratio 1 : 1, PAG/$ZnCl_2$ ratio 25 : 1) were carried out with 100 g of penta-acetyl-β-glucose the yield fell from 47 to 35%. Commercial powdered zinc chloride was used in all cases and crystallization was from 2 ml ethanol per g of reactants.

The author is grateful to Dr. R. Thomas for cooperation in parts of this work.

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A NEW SYNTHESIS OF 7,4′-DIMETHOXY-3-PHENYLCOUMARIN*

By R. B. Bradbury†

The striking similarity between the naturally occurring *iso*flavones, one of which (genistein, I) has been shown to have oestrogenic properties (Bradbury and White 1951), and 3-phenyl-4-hydroxycoumarin (II), which shows anti-vitamin-K activity, has been stressed by Mentzer (1953). This close relationship is further emphasized when one considers the possibility of addition of water across the double bond of an *iso*flavone (III) to give a 2-hydroxy*iso*flavanone (IV), followed by enolization to a 2,4-dihydroxy*iso*flav-3-en (V). It is well known that 2-hydroxy*iso*flavanones are sufficiently stable to be isolated as intermediates in the formic ester synthesis (Narasimhachari, Rajagopalan, and Seshadri 1953). In order to determine unequivocally the position of the double bond in certain *iso*flavens previously reported (Bradbury and White 1953) 7,4′-dimethoxy-3-phenylcoumarin was prepared as a reference compound for comparison of ultraviolet absorption measurements. The method of synthesis is new and should be generally applicable to compounds of this class. 3-Phenyl-4-alkyl-substituted coumarins can be prepared by the Pechmann condensation (Wawzonek 1951, p. 181), and 7,4′-dimethoxy-3-phenylcoumarin and related compounds by the Perkin reaction (Bhandari, Bose, and Siddiqui 1949), but the yields reported for the last procedure are poor. In the present work, 2,4-dimethoxybenzaldehyde (Adams and Montgomery 1924) was condensed with *p*-methoxybenzyl cyanide in the presence of sodium ethoxide according to the

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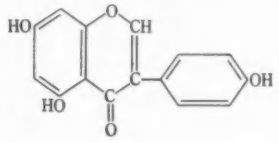
procedure of Niederl and Ziering (1942*a*, 1942*b*) to give 4,2′,4′-trimethoxy-α-cyanostilbene in 85 per cent. yield. Hydrolysis of the nitrile under acid or alkaline conditions proved unsuccessful, but refluxing with aluminium chloride in dry benzene caused demethylation and gave a water-soluble compound containing nitrogen and chlorine. This was evidently the imine-hydrochloride



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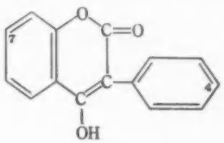

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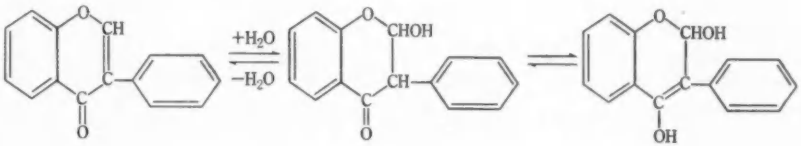
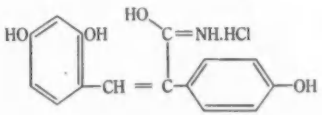


(III) (IV) (V)

(VI), since acetylation and methylation yielded respectively 7,4′-diacetoxy- and 7,4′-dimethoxy-3-phenylcoumarin, having properties in substantial agreement with those reported by Bhandari, Bose, and Siddiqui (loc. cit.) for these compounds. A number of suitably substituted coumarins show fluorescence in solution (Wawzonek 1951, p. 193), and 7,4′-dimethoxy-3-phenylcoumarin shows a characteristic violet fluorescence in alcohol.



(VI)

Experimental

All melting points are corrected. Microanalyses were carried out in the C.S.I.R.O. Microanalytical Laboratory.

(*a*) *4,2′,4′-Trimethoxy-α-cyanostilbene.*—2,4-Dimethoxybenzaldehyde (Adams and Montgomery loc. cit.) (18 g), and *p*-methoxybenzyl cyanide (16 g) were dissolved in ethanol (100 ml) and a solution of sodium ethoxide (from 2·5 g sodium) in ethanol (75 ml) added. After keeping in a water-bath at 50 °C for 1 hr, the cyanostilbene separated as long yellow prisms. Concentrated hydrochloric acid (11 ml) was added, the cyanostilbene brought into solution, the sodium chloride filtered, and the solution cooled. 4,2′,4′-Trimethoxy-α-cyanostilbene (85%; 27·2 g),

m.p. 137 °C, unchanged on recrystallization from benzene was obtained (Found: C, 73·5; H, 5·8; N, 4·9%. Calc. for $C_{18}H_{17}O_3N$: C, 73·2; H, 5·8; N, 4·7%). Attempts to hydrolyse the cyanostilbene by long refluxing with hydrochloric acid, or alcoholic sodium hydroxide, even in the presence of hydrogen peroxide were unsuccessful.

(*b*) *Demethylation and Hydrolysis with Aluminium Chloride.*—4,2',4'-Trimethoxy-α-cyanostilbene (9·5 g), powdered aluminium chloride (44 g), and dry benzene (100 ml) were refluxed for 16 hr, and poured into 20% aqueous hydrochloric acid cooled in ice. When the excess of aluminium chloride had reacted the mixture was warmed on a water-bath for ¼ hr, and the solid collected (6 g). Recrystallization from water gave yellow needles, m.p. 334 °C (decomp.) (Found: N, 4·3%. Calc. for $C_{15}H_{14}O_4NCl$: N, 4·6%). A qualitative test showed chlorine to be present.

(*c*) *7,4'-Diacetoxy-3-phenylcoumarin.*—The above imine-hydrochloride (0·5 g) and acetic anhydride (10 ml) were refluxed for 2 hr, poured into water, and the product recrystallized from acetic acid. Colourless needles (0·47 g), m.p. 216 °C (Found: C, 67·5; H, 4·2%; mol. wt. (Rast), 347. Calc. for $C_{19}H_{14}O_6$: C, 67·5; H, 4·2%; mol. wt., 338). Bhandari, Bose, and Siddiqui (loc. cit.) report m.p. 213–214 °C.

(*d*) *7,4'-Dimethoxy-3-phenylcoumarin.*—The imine-hydrochloride (0·99 g), dry acetone (100 ml), potassium carbonate (5 g), and dimethyl sulphate (2 g) were refluxed for 3 hr, filtered hot, and the inorganic salts extracted once with hot acetone. The combined filtrate on evaporation gave long colourless needles (0·65 g) which on recrystallization from acetone had m.p. 188–189 °C (Found: C, 72·5; H, 5·1; OMe, 22·0%. Calc. for $C_{17}O_{14}O_4$: C, 72·3; H, 5·0; OMe, 21·8%). Bhandari, Bose, and Siddiqui (loc. cit.) report m.p. 182 °C. The ultraviolet spectrum of this compound has been reported elsewhere (Bradbury and White 1953).

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N-CHLOROACETYLISATIC ACID*

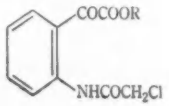
By R. JOHNSTONE† and J. R. PRICE†

Huntress and Bornstein (1949) report that *N*-chloroacetylisatin is yellow, but crystallizes from methanol in a colourless form containing 1 mole of solvent. For the following reasons it is considered that the colourless compound is the methyl ester of *N*-chloroacetylisatic acid (I, R $=CH_3$) rather than a hemiketal type (II) analogous to ninhydrin :

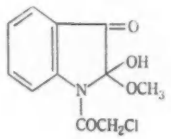
(i) The methanol cannot be replaced by crystallization of the methanol adduct from other alcohols or from water, nor removed by prolonged distillation with toluene.

(ii) Whereas *N*-chloroacetylisatin dissolves in concentrated sulphuric acid to an orange solution, the methanol adduct gives a colourless solution from which it is precipitated unchanged by pouring into water. Ninhydrin dissolves in concentrated sulphuric acid giving a red solution, the colour of which is due to unhydrated triketohydrindene (MacFadyen 1950).

(iii) The addition of water to *N*-chloroacetylisatin is only achieved with difficulty and the product, which is acidic, is evidently *N*-chloroacetylisatic acid.



(I)



(II)

Methyl *N*-chloroacetylisatic ester is reconverted to *N*-chloroacetylisatin by heating with acetic anhydride. The ethyl ester (I, R $=C_2H_5$) has been prepared.

Experimental

Melting points are corrected. Microanalyses were carried out in the C.S.I.R.O. Microanalytical Laboratory.

(*a*) *N-Chloroacetylisatic Acid.*—*N*-Chloroacetylisatin (1 g) dissolved in cold 1% sodium hydroxide giving a red solution which changed rapidly to yellow. Acidification with cold 5% hydrochloric acid precipitated a pale yellow solid (0·54 g), which was filtered off immediately, and crystallized three times from ethyl acetate. *N*-Chloroacetylisatic acid separated as almost

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colourless needles, m.p. 167–168 °C (Found: C, 49·9; H, 3·3%. Calc. for $C_{10}H_8O_4NCl$: C, 49·7; H, 3·3%). Isatin (m.p. and mixed m.p. 203–204 °C) separated from the aqueous acid filtrate on standing. *N*-Chloroacetylisatic acid dissolves in aqueous sodium bicarbonate with evolution of carbon dioxide.

(*b*) *Solvolysis of N-Chloroacetylisatin.*—*N*-Chloroacetylisatin was recovered unchanged (yellow needles, m.p. and mixed m.p. 210–211 °C) after boiling with ethyl acetate or acetone, or on pouring a solution in concentrated sulphuric acid into water. Refluxing for 1 hr with 50% aqueous acetone (0·5 g *N*-chloroacetylisatin in 40 ml) and evaporating to 15 ml gave *N*-chloroacetylisatic acid (0·26 g), m.p. and mixed m.p. 167–168 °C after recrystallization from water. Isatin (0·12 g) was recovered from the filtrates. *N*-Chloroacetylisatin dissolved slowly in boiling water and after refluxing the solution for 3 hr the only product was isatin (0·13 g from 0·2 g chloroacetyl compound), m.p. and mixed m.p. 203–204 °C.

The methyl ester of *N*-chloroacetylisatin, obtained as colourless needles, m.p. 83–83·5 °C after boiling a solution of *N*-chloroacetylisatin in methanol, was unchanged by crystallization from water, ethanol, *iso*propanol, chloroform, ethyl acetate, benzene, or ether. It separated unchanged from a toluene solution which had been concentrated by slow distillation for 1½ hr, but was converted to *N*-chloroacetylisatin, m.p. and mixed m.p. 209–210 °C by refluxing for 1 hr with acetic anhydride. The methyl ester dissolved in concentrated sulphuric acid to a colourless solution from which it was precipitated unchanged on pouring into water.

The *ethyl ester* of *N*-chloroacetylisatic acid, prepared by boiling an ethanol solution of *N*-chloroacetylisatin, crystallized from light petroleum as cream plates, m.p. 64–64·5 °C (Found: C, 53·9; H, 4·6%. Calc. for $C_{12}H_{12}O_4NCl$: C, 53·4; H, 4·5%).

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